Guide Book Ver. 3.0

All through,

ExiProgen™

Automatic Protein Synthesizer

From Genes to Proteins
in 6 hours

The world's first
fully-automated
Protein Synthesis and
Nucleic Extraction system

BIONEER
Innovation • Value • Discovery

All through, ***ExiProgen™***

Guide Book

BIONEER

Innovation • Value • Discovery

How to use this

Guide Book

This is a guide book which provides information about BIONEER Corperation's products related with *ExiProgen*™, It is a fully-automatically instrument performing various steps for protein synthesis, including nucleic acid preparation, recombinant protein expression and purification.

Guide structure

Go straight for your needs!

Contact Us

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1588-9788 sales@bioneer.co.kr www.bioneer.com

Contents

Fully-automated instrument for the synthesis of protein:
Try to research with *ExiProgen*™ a wide range of your proteins conveniently.

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Workflow of Protein Synthesis

From Sample to Proteins
using BIONEER's products

Nucleic acid Extraction and Purification

Template Preparation (2-step PCR)

PCR/Gel Purification

Protein Synthesis (Expression and Purification)

Following the workflow below, the use of instrument *ExiProgen*™ will be the most efficient and of highest value for protein research. Please also note that the ability of *ExiProgen*™ is not limited to the below functions, but more can be done.

Extract/purify nucleic acids in a fully-automated process

- Instrument: *ExiProgen*™
- Kit: *ExiPrep*™ Kit Series

Prepare linear template DNA manually

- Kit: *ExiProgen*™ ProXpress PCR Template Kit

OR

ExiProgen™ Protein Expression Optimization Kit

Conduct PCR/Gel purification

- Instrument: *ExiProgen*™
- Kit: *MagListo*™ PCR/Gel Purification Kit (manual type)

+

ExiProgen™ Consumable SET (for automation)

Synthesize/purify target proteins in a fully-automated process

- Instrument: *ExiProgen*™
- Kit: *ExiProgen*™ Kit Series

Part. I

About *ExiProgen*™

Chapter 1 -
Automatic Protein Research Instrument, *ExiProgen*™

1.1. Introduction

ExiProgen™ is a novel instrument of BIONEER Corporation's to carry out various steps for synthesizing recombinant proteins, such as nucleic acid extraction, PCR purification, protein expression and purification from lysates.

Nucleic acid extraction using *ExiProgen*™ is performed with the related kits (*ExiPrep*™ Kit series). It allows customers to obtain nucleic acid from sample within an hour to 2 hours and covers more than 7 kinds of biological sources from different species such as blood, animal and plant tissues, bacteria, etc.

And also, PCR purification is conducted with *MagListo*™ PCR/Gel Purification Kit and *ExiProgen*™ Consumable set. With *MagListo*™ and *ExiProgen*™ products lines, *ExiProgen*™ can easily prepare nucleic acid samples needed for biotechnology experiment.

Protein synthesis in *ExiProgen*™ is automatically performed in order of cell-free protein expression system. It can synthesize up to 16 different proteins at a time and takes at least 6 hours. Therefore, it would be a valuable tool for fast screening of mutant proteins and production of recombinant proteins. Besides, BIONEER provides various protein synthesis kits (*ExiProgen*™ Kit series) depending on target protein types and quantities.

Protein purification from cell lysates is available in *ExiProgen*™, apart from protein synthesis step and is conducted with Ni-NTA beads, which is combined with His-tag, or Protein A, Protein L and Protein G. In case of protein purification, customers require to insert certain sequence into the template DNA (plasmid or PCR product). *ExiProgen*™ can carry out a series of experiments for recombinant proteins synthesis quickly and conveniently. It is also versatile to compatible for performing each step of molecular experiments. Refer to this guide book when you select desired products or learn more about the related products.

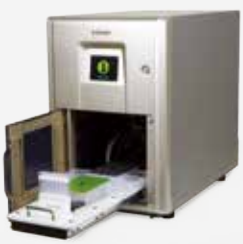
Automatic Protein Research Instrument, *ExiProgen*™

Nucleic Acid Extraction
Automatically isolate genomic DNA from various biological samples

PCR Purification
Automatically purify PCR product using magnetic nanobead

Protein Synthesis
Takes only 6 hours from genes to proteins

Protein Purification
Automatecally purify proteins through affinity tagging



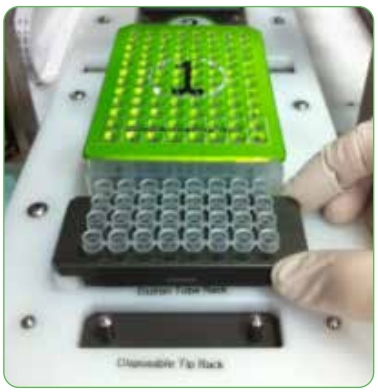
1.2. *ExiProgen*™

The following are several unique features of *ExiProgen*™.

Optimized Protocols with Specialized Properties

ExiProgen™ comes with many different protocols for protein synthesis/purification & nucleic acid extraction, each optimized to unique features of individual sample. BIONEER continues to update the protocols to keep improving the capability of *ExiProgen*™. The updated protocols are readily available on BIONEER's website and can be downloaded to your computer for installation to *ExiProgen*™ via TCP/IP.

Temperature-Controlled Block Installed



The temperature range of the temperature-controlled block is between 4°–90°C, allowing protein synthesis reaction to be performed at an optimized temperature and the final purified protein to be stored at an optimized low temperature.

Magnet & Heating Block Installed

ExiProgen™ provides a magnet and a heating block for protein or nucleic acid purification. Magnetic field is applied to evenly attract or disperse magnetic particles when washing or eluting molecules. The temperature range is between 30–90°C. The temperature can also be finely controlled and maintained stable during purification. This also helps evaporating away any residual ethanol after nucleic acid purification (KR101555534B1)*.

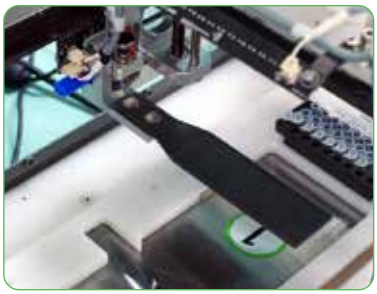
Patent

* *ExiProgen*™ includes two patented technologies:

1. KR101555534B1 – Automatic cell-free protein synthesis method and instrument
2. WO2013032174A3 – Protein expression and extraction method using protein synthesis kit and automatic extraction instrument

Minimized Cross-contamination

Contamination Shield is located underneath the disposable filter tip, which prevents well-to-well contamination during automated pipetting and liquid handling.



Touch Screen

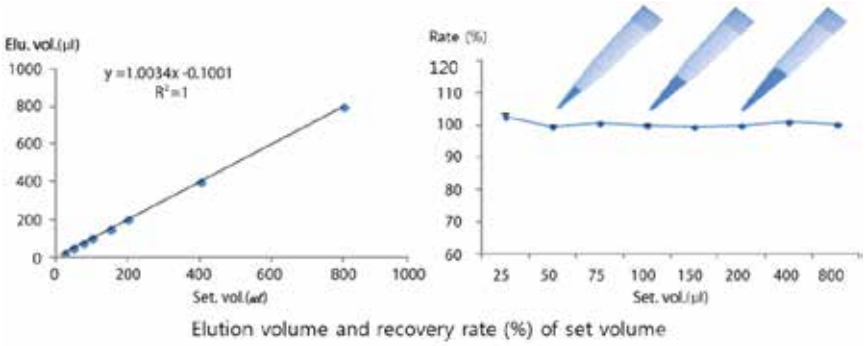
3.5" touch screen can be interactively used for selecting protocols, choosing elution volumes, etc. The touch screen also displays the progresses of protein synthesis and nucleic acid purification protocols.

UV Sterilization Lamp

ExiProgen™ has a built-in strong short wavelength UV lamp for chamber sterilization, that runs before or after running the machine. This feature helps to prevent cross-contamination between each step.

Accurate Pipette Volume

ExiProgen™ is equipped with highly accurate syringe motor that minimizes pipetting error during liquid transfer. It accurately pipettes any volume between 25 to 1,000 µl.



Elution volume and recovery rate (%) of set volume

Specification

Dimension(cm)	32 (W) × 53.5 (D) × 50 (H)
Weight	27 kg
Temperature range	15-35℃
Humidity range	20-80%, no condensation
Operating system	Stand-alone
User interface	320 × 240 touch screen graphic LCD
Input voltage	100-240 VAC
Frequency	50/60 Hz
UV sterilization	15 minute cycler
Communications	TCP/IP
Heat block	40-90℃

Components

ExiProgen™

ExiProgen™
User Guide

Disposable Tip Rack

Elution Tube Rack

Reaction Block
(For Protein Synthesis)

6 Hole Punch

Waste Tray

Contamination Shield

Adaptor

Multi Puncher (Option)

Protection Cover
Separation Tool

Setup Tray

Chapter 2 - Value of *ExiProgen*™

2.1. Value of *ExiProgen*™

In this part of the Guide Book, the value of *ExiProgen*™ will be introduced according to the following order:

- Patented SECF System
- Bulk Protein Synthesis
- Protein Expression Optimization
- Fluorescent Protein Synthesis
- High Molecular Weight Protein Synthesis
- pET Vector Protein Synthesis

Introduction

In the post-genomic era, where vast DNA sequence information across many organisms has accumulated, identifying protein structure, function and protein-protein interactions is crucial to understand cellular and metabolic process as well as the cause of diseases at the molecular level. Protein expression is necessary to study the functional activity of proteins and the typical method for protein expression is *in vivo* expression using cells. This method involves the transfection of recombinant DNA into the host cell, cell culture and subsequent lysis and purification. However, protein expression by cell culturing is a time-consuming process, and it is not easy to synthesize a number of proteins at the same time.

To overcome this shortcoming, cell-free protein synthesis (*in vitro* transcription/translation) methods have been developed. Cell-free methods use cell extracts combined with ingredients essential for protein synthesis and reactions are performed *in vitro*. When recombinant DNA coding for the protein of interest is added to reaction mixture and incubated at certain conditions, the desired protein is synthesized. Because this method does not require a separate cell line selection step, it is able to yield different types of protein in a single experiment within a short period of time, increasing the variety and throughput of protein synthesis. *In vitro* protein expression can also synthesize proteins that are difficult to express due to cellular toxicity effects, etc.

In the following few pages, applications of the instrument *ExiProgen*™ will be discussed for different types of protein synthesis and with different purposes.

ExiProgen™'s patented technology:

Patented SECF System

Synthesis of Periplasmic AppA Protein using *ExiProgen*™ Automated Protein Synthesis Equipment

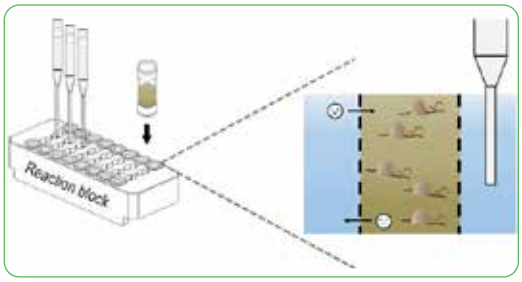
Abstract

Easy and rapid production of recombinant protein has been possible since the implementation of cell-free protein synthesis (CFPS) to an automatic system. By controlling the folding environment, proteins with disulfide bonds in *E. coli* base CFPS can be expressed. In this study, we synthesized periplasmic AppA protein having four disulfide bonds with *ExiProgen*™, an automatic protein synthesis instrument, and evaluated their protein functions by measuring the enzyme activities. Stepwise exchange cell-free (SECF) protein expression method was used in an attempt to increase the final protein yields.

Kit: *ExiProgen*™ EC-Disulfide Protein Synthesis Kit [K-7330]
Purpose: Synthesis of periplasmic AppA protein having four disulfide bonds

Description

Stepwise Exchange Cell Free (SECF) System



Scheme 1. Protein expression in SECF system.
Dialysis tubes, filled with reaction mixtures, are fixed in the reaction block. Transcription and translation take place in the tubes. Energy sources and substrates are continuously supplied via semi-permeable membranes, whereas inhibitory byproducts are diluted via diffusion through the same membrane.

Synthesis of disulfide proteins with *ExiProgen*™

Scheme 2. The process of synthesizing disulfide proteins with *ExiProgen*™.
Protein expression takes about 26 hours in 26℃ by SECF system, and purification (using Ni-NTA beads) and dialysis (to exchange buffer) are carried out afterwards, making the whole process 34 hours long in total.

Experimental Results

Synthesis of AppA protein with *ExiProgen*™

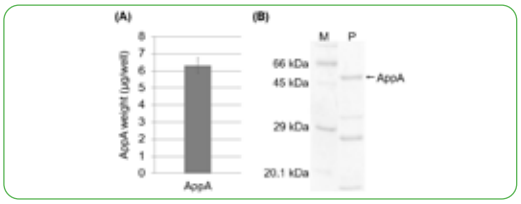


Figure 1. Protein analysis of AppA protein.

Enzymatic activity of AppA synthesized with *ExiProgen*™

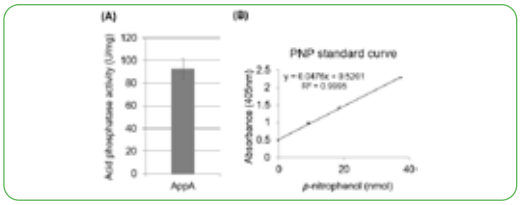


Figure 2. Acid phosphatase activity assay of synthesized AppA was determined as 92.5 U/mg.

Conclusion

- To increase the final yield of synthesized disulfide proteins with enzymatic activities, we introduced SECF system while adding glutathione buffer and chaperons.
- Even with 4 disulfide bonds, active AppA proteins were synthesized within 34 hours, including the expression, purification and dialysis step; all the processes were done automatically using *ExiProgen*™.
- The results show that the automatic protein synthesis system has great potential in both the production of vaccines and the screening of pharmaceutical proteins, both in terms of time and yield.

HIT!

Protein Synthesis up to 10 mg protein: **Bulk Protein Synthesis**

Simple method for screening and production of antibody using *ExiProgen*™

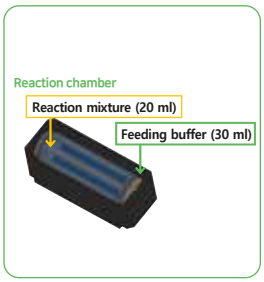
Abstract

In this study, scFv, scFv-Fc antibodies and other derivatives were designed and evaluated after expression or purification. The best ratio of molecular chaperone proteins for disulfide bond formation was also determined for each antibody or antibody derivative. Additionally, *ExiProgen*™ EC-Bulk Protein Synthesis Kit was designed to produce 10-fold larger amount of scFv-Fc or other antibody derivatives than *ExiProgen*™ EC-Disulfide Protein Synthesis Kit. Therefore, it is concluded that *ExiProgen*™ would be a valuable tool for screening and production of antibody and its derivatives.

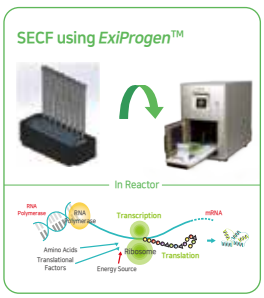
Kit: *ExiProgen*™ EC-Bulk Protein Synthesis Kit [K-7340]
Purpose: Bulk Synthesis of Proteins
Sample Type: scFv, scFv-Fc antibodies and other derivatives

Description

Scheme 1. Reaction mixture for bulk synthesis of proteins.
The reactor is filled with reaction mixture and covered with semi-permeable membrane.



Scheme 2. Protein expression in Stepwise Exchange Cell-Free (SECF) system.
Reactor, filled with reaction mixture including PCR products or plasmid DNA as template, was installed at reaction chamber. Then reaction chamber was filled with 30 ml of feeding buffer.



Scheme 3. The process of disulfide protein synthesis with *ExiProgen*™.
After kit setup, reaction was started under pre-installed protocol. During the reaction extra volume of feeding buffer was supplied 12 times into reaction chamber to increase protein expression according to SECF mode.

Experimental Results

A. scFv

A-1 Expression sample A-2 Purification sample

Figure 1. One of four screening experiments for an optimized production of antibody fragments.

A. DS vs. Bulk Kit

B. Bradford assay

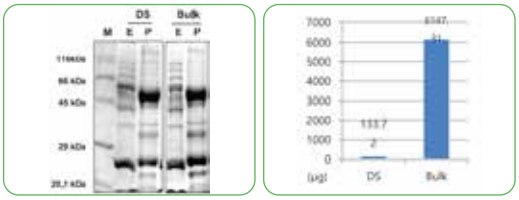


Figure 2. Comparison of protein production between *ExiProgen*™ EC-Disulfide Protein Synthesis Kit and *ExiProgen*™ EC-Bulk Protein Synthesis Kit.

Conclusion

- Screening of chaperone protein was performed to determine the best value in their ratio for antibody fragment expression.
- New device for a 10-fold higher yield of protein production in *ExiProgen*™ was designed. This device was able to harvest about 40-fold increased amount of protein compared to the current method.
- Antibody fragments were expressed then purified with the instrument *ExiProgen*™ using *ExiProgen*™ Consumable SET and various magnetic beads – Protein G, A or L – according to their regions.

HIT!

Solution for Successful Protein Expression : **Protein Expression Optimization**

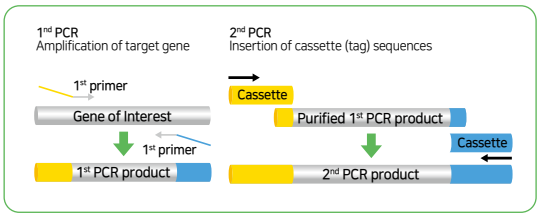
Introduction

ExiProgen™ Protein Expression Optimization Kit generates linear template DNA that contains six different types of tag sequences, which can be used to synthesize various tags for maximizing the target protein expression. This enables expressing proteins that were once difficult to express using the standard *ExiProgen*™ protein synthesis instrument (EC Protein Synthesis Kit). Furthermore, each of the template DNA contains TEV cleavage sites, allowing removal of tags after the protein synthesis if needed. In this study, we demonstrated the efficiency of this kit by applying it to protein that could not be expressed using the typical protein expression system.

Kit for template DNA preparation : *ExiProgen*™ Protein Expression Optimization Kit [K-7410]
Kit for cell-free protein synthesis : *ExiProgen*™ EC Protein Synthesis Kit [K-7300]
ExiProgen™ EC-Tagfree Protein Synthesis Kit [K-7320]
Purpose: Expression of difficult-to-express proteins.

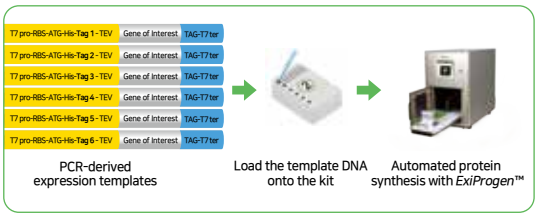
Description

Preparation of DNA with Expression Enhanced Tags



Scheme 1. Diagram of the two-step PCR to preparetemplate DNA.

Synthesis of Target Proteins with *ExiProgen*™



Scheme 2. The process of synthesizing proteins with *ExiProgen*™.

Experimental Results

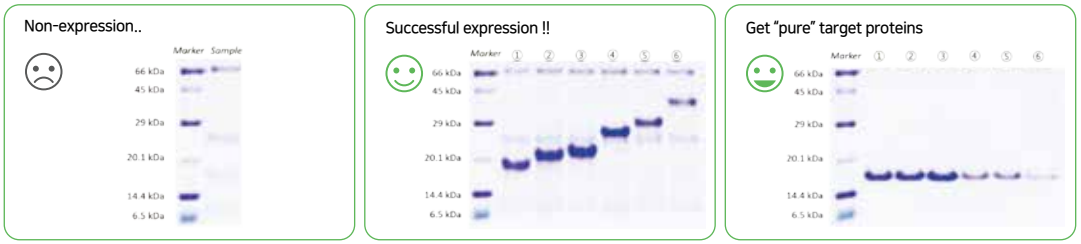


Figure 1. Synthesis of a difficult-to-express protein from various expression templates.
Marker: Protein Size marker, Sample: Non-tagged protein (18 kDa), Lane 1-6: Synthesized proteins from tag 1-6 inserted genes.

Conclusion

- Cell-free expression templates were rapidly prepared by the two-step PCR method and screened through a fully automated *in vitro* protein synthesizer.
- The non-expression protein was successfully expressed and tag-removed target proteins were also obtained using *ExiProgen*™ EC-Tagfree Protein Synthesis Kit.
- *ExiProgen*™ Kits saved the time required for expression template preparation and protein synthesis, and allowed more proteins to be easily produced in an automated cell-free system.

ExiProgen™ : Fully Automated Protein Synthesis instrument

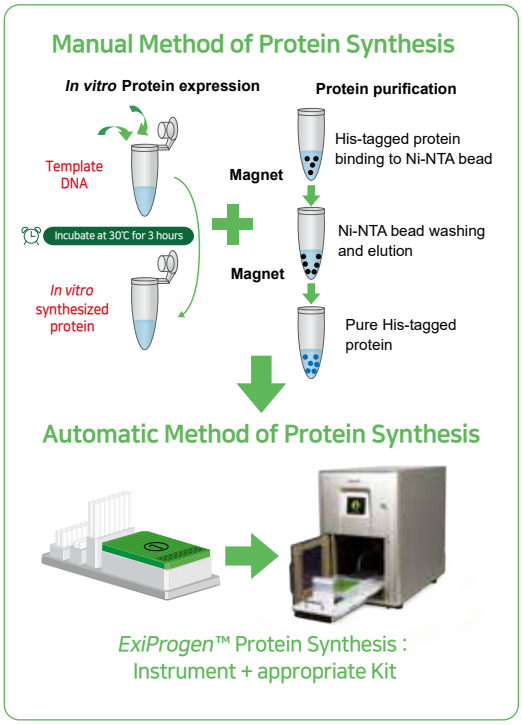
Fluorescent Protein Synthesis

Introduction

ExiProgen™ Protein Synthesis instrument brings automation to *in vitro* protein expression and magnetic bead-based His-Tag affinity purification. Up to 16 highly pure proteins can then be obtained in less than 6 hours. The workflow is simple; 1) template DNA preparation, 2) template DNA and components loading onto the instrument, and 3) starting the instrument. The related kit contains optimized *E. coli* extract which contains T7 RNA polymerase and ribosomes, all other required components such as amino acids and an energy source for efficient *in vitro* transcription and translation. The kit also contains Ni-NTA magnetic beads for a fast and effective purification of expressed His-tagged proteins. With the instrument and the kit, up to 16 different kinds of highly pure proteins can be obtained from a single experiment within 6 hours.

Kit: *ExiProgen*™ EC Protein Synthesis Kit [K-7300]
Purpose: Cell-Free Protein Synthesis of Fluorescent Proteins
Sample Type: pBIVT1-AcGFP vector SfGFP expression vector, RFP expression vector

Description



Scheme 1. Comparison of procedures between manual and automatic protein expression and purification.
Upper panel: Manual method of protein expression and purification: sequential and time-consuming.
Lower panel: Automated method of protein expression and purification: setup and walk-away.

Experimental Results

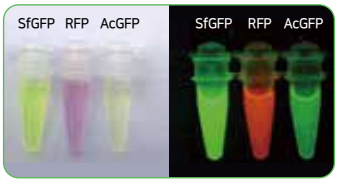


Figure 1. Color (left) and fluorescence (right) from eluted protein samples, SfGFP, RFP, and AcGFP, synthesized with *ExiProgen*™.

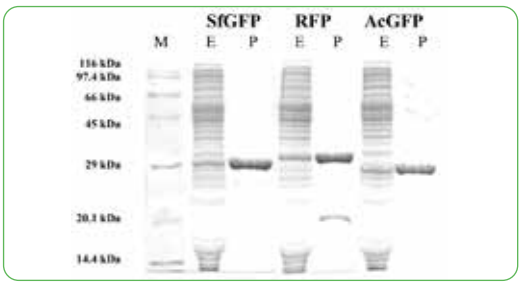


Figure 2. Each of expression and purification sample of SfGFP, RFP and AcGFP was run on 12% SDS-PAGE gel.
Lane M: Protein Size Marker
Lane E: Expression sample (not purified)
Lane P: Purified sample

Conclusion

Fluorescent proteins were successfully synthesized in a cell-free manner and purified with Ni-NTA beads using *ExiProgen*™. This automated, easy-to-use, and rapid protein synthesis instrument has many potential applications in various research fields including the study of protein function, protein-protein interaction, protein structure as well as enzyme engineering and in biofuel research.

For an original application note, visit the product page by searching [A-5041] in BIONEER's website (eng.bioneer.com)

ExiProgen™ : Fully Automated Protein Synthesis instrument

High Molecular Weight Protein Synthesis

Introduction

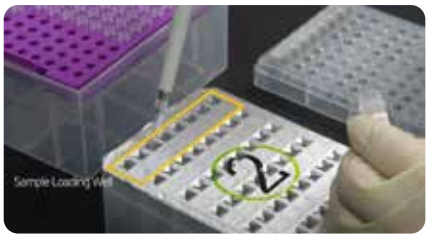
Cell-free protein synthesis is a common method to synthesize a desired protein in a rapid and efficient manner. It is accomplished by adding template DNA (containing the coding region of the protein of interest) into a single tube which contains cell extracts and other ingredients essential for protein synthesis. Because this method does not require a separate cell line selection step, it is able to yield diverse types of protein in a very short period of time when compared to *in vivo* protein expression. *ExiProgen*™ Protein Synthesis instrument brings automation to *in vitro* protein expression and magnetic bead-based His-Tag affinity purification. Up to 16 highly pure proteins can then be obtained in less than 6 hours. With the instrument, up to 16 different kinds of highly pure proteins can be obtained from a single experiment within 6 hours.

Kit: *ExiProgen*™ EC Protein Synthesis Kit [K-7300]
Purpose: Cell-Free Protein Synthesis of Fluorescent Proteins
Sample Type: *Bacillus megaterium* 3 (BM3) expression vector

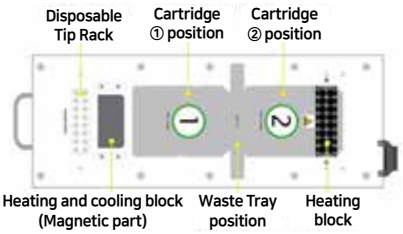
Description

Procedure of using *ExiProgen*™ and related-kits

1) Dispense template DNA



2) Load components onto the instrument



3) Run the instrument

Experimental Results

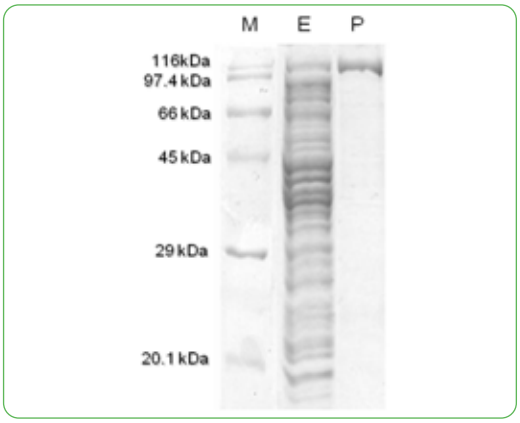


Figure1. Expressed and Purified samples run through SDS-PAGE gel analysis. *ExiProgen*™ is able to synthesize proteins as large as 120 kDa.
Lane M: Protein Size Marker
Lane E: Unpurified expression sample
Lane P: Purified sample

Conclusion

BM3 protein was successfully expressed and purified in a cell-free manner and purified with Ni-NTA beads using *ExiProgen*™, indicating that it is able to synthesize proteins as large as 117 kDa. This automated, easy-to-use, and rapid protein synthesis instrument has many potential applications in various research fields including the study of protein function, protein-protein interaction, protein structure as well as enzyme engineering.

For an original application note, visit the product page by searching [A-5041] in BIONEER's website (eng.bioneer.com)

ExiProgen™ : Fully Automated Protein Synthesis instrument

pET Vector Protein Synthesis

Introduction

Protein expression using pET expression system is typically accomplished *in vivo* using *E. coli* cells that express T7 polymerase. This method involves the transfection of the pET recombinant DNA into the host cell, selecting a cell line, cell culture and subsequent lysis and purification. However, protein expression by cell culture is a time-consuming process; hence, cell-free protein synthesis is used to yield desired protein in an efficient manner by adding template DNA containing the coding region of protein of interest to a cell extract. A major issue is that when using both *in vivo* and *in vitro* expression systems, *in vitro* protein expression vectors do not work well *in vivo*, and vice versa. In this study, we demonstrate that at least some of the vectors in the pET series can be used as template DNA for the cell-free protein synthesis with *ExiProgen*™ Protein Synthesis instrument.

Kit: *ExiProgen*™ EC Protein Synthesis Kit [K-7300]
Purpose: Expression of pET Vectors *in vivo*
Sample Type: pET protein expression vectors

Experimental Results

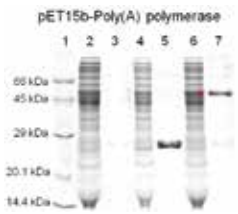


Figure 1. pET15b-poly(A) polymerase was used as a template DNA for the synthesis of poly(A) polymerase.

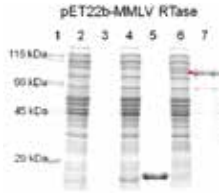


Figure 2. pET22b-MMLV RTase was used as a template DNA for the synthesis of MMLV RTase.

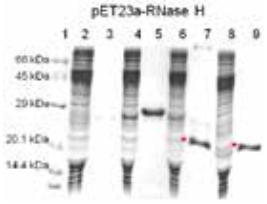


Figure 3. pET23a-RNase H was used as a template DNA for the synthesis of poly(A) polymerase

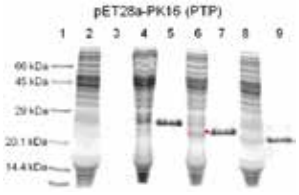


Figure 4. pET28a-PK16 (PTP) was used as a template DNA for the synthesis of PK16 (PTP)

Lane 1: Protein Size Marker
Lane 2: Expression sample of negative control
Lane 3: Purified sample of negative control
Lane 4: Expression sample of positive control (pBIVT2-CAT)
Lane 5: Purified sample of positive control (pBIVT2-CAT)
Lane 6: Expression sample
Lane 7: Purified sample

Conclusion

Four pET vectors were successfully expressed and purified in a cell-free manner and purified with Ni-NTA beads using *ExiProgen*™. Due to the automation, simplicity of use, and rapidness of protein synthesis, *ExiProgen*™ has the potential in applying to various research fields including identification of protein function, protein-protein interaction study, high throughput synthesis as well as enzyme engineering.

For an original application note, visit the product page by searching [A-5041] in BIONEER's website (eng.bioneer.com)

2.2. Research literature published by customers using *ExiProgen*™

Removal of the mechanoprotective influence of the cytoskeleton reveals PIEZO1 is gated by bilayer tension

Description

Research Institute	Victor Chang Cardiac Research Institute
Publisher	Nature Communications
Aim of Research	To examine whether the mechanosensitive ion channel PIEZO1 is activated by force-transmission through the bilayer.
Use of Instrument	*ExiProgen*™ / To automatically synthesize and purify protein.
Use of *ExiProgen*™ Kit	*ExiProgen*™ EC Protein Synthesis Kit [Cat. No. K-7300]
Target Protein	MscL-G22S Protein
Applied field	Mechanosensation-related Neuroscience (Proteoliposome Preparation)
Further applications	To study functions of transporter assay, ion channel characterization, and membrane proteins (e.g. ion channel, transporter) that is usually difficult to express.

Role of *ExiProgen*™

In the study by Cox *et al.*, the instrument *ExiProgen*™* and the Kit were used to express and purify MscL-G22S protein**, which was used as an integral membrane protein for MscL proteoliposomes reconstitution.

Furthermore, chemicals and additives of choice*** were added onto the expression and purification wells as desired.

In the literature, 10 µg of template plasmid was added onto the Kit. After loading the kit and its components, the instrument setup is done and the protocol is selected. Then, the instrument starts running.

*** Notes:** * The instrument *ExiProgen*™ is a fully-automated instrument that synthesizes protein in a cell-free manner, and purifies the synthesized protein using Ni-NTA affinity.

** MscL-G22S Protein is a mechanosensitive channel of *E. coli* (MscL) with Glycine residue in the hydrophobic gate of the protein mutated to a serine (MscL-G22S).

*** *ExiProgen*™ EC Protein Synthesis Kit provides all necessary components for protein synthesis and purification, and additives like chemicals and buffer of choice can also easily be added.

Literature Information

Cox, C. D., Bae, C., Ziegler, L., Hartley, S., Nikolova-Krstevski, V., Rohde, P. R., ... & Martinac, B. (2016). Removal of the mechanoprotective influence of the cytoskeleton reveals PIEZO1 is gated by bilayer tension. *Nature communications*, 7(1), 1-13.

2.2. Research literature published by customers using *ExiProgen*™

Automated Cell-Free Multiprotein Synthesis Facilitates the Identification of a Secretory, Oligopeptide Elicitor-Like, Immunoreactive Protein of the Oomycete *Pythium insidiosum*

Description

Research Institute	Faculty of Medicine, Ramathibodi Hospital, Mahidol University
Publisher	mSystems
Aim of Research	To demonstrate that cell-free protein synthesis is useful in producing multiple proteins for functional studies and to identify potential target for diagnosis and treatment of pythiosis.
Use of Instrument	*ExiProgen*™ / To automatically synthesize and purify protein.
Use of *ExiProgen*™ Kit	*ExiProgen*™ ProXpress PCR Template Kit [Cat. No. K-7400], *ExiProgen*™ EC Protein Synthesis Kit [Cat. No. K-7300]
Target genes	24 genes of the oomycete *Pythium insidiosum*
Applied field	Therapeutic Protein Research (Screening for immunoreactivity)
Further applications	For screening to develop therapeutic proteins that can act against diseases and be used like vaccines, diagnostic / therapeutic agents.

Role of *ExiProgen*™

ExiProgen™ ProXpress PCR Template Kit* was used to prepare template DNA using genomic DNA (10 ng) for cell-free protein synthesis, without cloning process but in a two-step PCR reaction.

Up to 16 proteins were simultaneously expressed and purified in a single run of *ExiProgen*™, using *ExiProgen*™ EC Protein Synthesis Kit**.

In this study, among the 24 genes, 18 proteins were successfully synthesized with protein concentration between 92-387 μg/ml.

*** Notes:** *The Kit contains all required reagents for template preparation, including upstream and downstream cassettes and primers.

**The Kit contains all required reagents for protein synthesis and purification, which includes 2 cartridges (which are prefilled with the required reagents), *E. coli* extract, plasticwares, etc.

Literature Information

Sae-Chew, P., Rujirawat, T., Kumsang, Y., Payattikul, P., Lohnoo, T., Yingyong, W., ... & Krajaejun, T. (2020). Automated Cell-Free Multiprotein Synthesis Facilitates the Identification of a Secretory, Oligopeptide Elicitor-Like, Immunoreactive Protein of the Oomycete P*ythium insidiosum. Msystems, 5*(3), e00196-20.

2.2. Research literature published by customers using *ExiProgen*™

Highly efficient genome editing by CRISPR-Cpf1 using CRISPR RNA with a uridinylate-rich 3′-overhang

Description

Research Institute	Genome Editing Research Center, Korea Research Institute of Bioscience & Biotechnology (KRIBB)
Publisher	Nature Communications
Aim of Research	To examine the most efficient indel efficiency of engineered CRISPR-Cpf1 system and compare it with the existing CRISPR-Cas9 system.
Use of Instrument	*ExiProgen*™ / To automatically synthesize and purify protein.
Use of *ExiProgen*™ Kit	*ExiProgen*™ EC Protein Synthesis Kit [Cat. No. K-7300]
Target Protein	AsCpf1 Protein
Applied field	Genome editing (CRISPR-Cas system)
Further applications	For research on fundamentals of CRISPR-Cas system. The existing CRISPR-Cas system relies on the current Cas9 protein-based modification to increase the efficiency, or on finding of a novel nuclease which might show higher efficiency.

Role of *ExiProgen*™

In the study by Bavi *et al.*, the instrument *ExiProgen*™* was used to synthesize and purify the recombinant AsCpf1 protein.

1 μg of the gene construct was added onto the reaction mixture**. The reaction tubes and the other components were then installed onto the instrument. After proper Protocol No. selection, *ExiProgen*™ was started.

The activity of the synthesized AsCpf1 protein was then compared and it is represented to be higher than SpCas9 activity.

*** Notes:** * The instrument *ExiProgen*™ is a fully-automated instrument that contains lots of protocols highly optimized for each function, and provides separate or combined running protocols.

** *ExiProgen*™ EC Protein Synthesis Kit contains all required reagents to prepare reaction mixture, which include T7 RNA polymerase, ribosome, tRNAs, enzymes, amino acids, rNTPS, salts, energy sources, etc.

Literature Information

Bin Moon, S. U., Lee, J. M., Kang, J. G., Lee, N. E., Ha, D. I., Kim, D. Y., ... & Kim, Y. S. (2018). Highly efficient genome editing by CRISPR-Cpf1 using CRISPR RNA with a uridinylate-rich 3′-overhang. *Nature communications*, 9(1), 1-11.

2.2. Research literature published by customers using *ExiProgen*™

Treatment of low-strength ammonia wastewater by single-stage partial nitritation and anammox using upflow dual-bed gel-carrier reactor (UDGR)

Description

Research Institute	School of Urban and Environmental Engineering, Ulsan National Institute of Science and Technology (UNIST)
Publisher	Bioresource Technology
Aim of Research	To investigate the effect of single-stage partial nitritation and anammox treatment (S-PNA), (targeting low-strength ammonia wastewater) on nitrogen removal efficiency (NRE).
Use of Instrument	*ExiProgen*™ / To automatically extract Total DNA
Use of *ExiProgen*™ Kit	*ExiPrep*™ Plus Bacteria Genomic DNA Kit [Cat. No. K-4214]
Applied field	Environmental Engineering (Ammonia Wastewater Treatment)
Further applications	For research on the effect of biomass enrichment on microbial community, which might yield other means of sewage treatment, or help study the evolutionary mechanism of microbial community.

Role of *ExiProgen*™

The Kit* was used to extract community DNA of municipal wastewater (Sc) using the instrument *ExiProgen*™**.

The aliquot of Sc was centrifuged and the supernatant decanted. Then, the pelleted biomass underwent repeated resuspending and decanting, and the final resuspension (200 µl) was prepared and loaded onto *ExiProgen*™.

The extracted DNA was collected as eluted in 100 µl of elution buffer***, and stored at -20℃ until use.

*** Notes:** * *ExiProgen*™ performs both NA extraction and protein synthesis. *ExiPrep*™ Kit series is responsible for nucleic acid (NA) extraction, while *ExiProgen*™ Kit series is responsible for protein synthesis and purification.

** The instrument *ExiProgen*™ provides a walk-away automation system that extracts different types of nucleic acids (NA).

*** *ExiPrep*™ Plus Bacteria Genomic DNA Kit contains all required reagents for nucleic acid extraction, which includes required buffers, plastic wares, etc.

Literature Information

Jo, Y., Cho, K., Choi, H., & Lee, C. (2020). Treatment of low-strength ammonia wastewater by single-stage partial nitritation and anammox using upflow dual-bed gel-carrier reactor (UDGR). *Bioresource Technology, 304,* 123023.

List of Literatures published by customers using *ExiProgen*™

Year	Purpose	Title of paper	Publisher
2022	Protein Synthesis, Purification	The Arabidopsis cyclophilin CYP18-1 facilitates PRP18 dephosphorylation and the splicing of introns retained under heat stress.	The Plant Cell
2022	NA Extraction	Seroprevalence of SARS-CoV-2 antibodies in healthcare workers at a tertiary care hospital in Riyadh, Saudi Arabia.	Microorganisms
2021	Protein Synthesis, Purification	Immunological Cross-Reactivity of Proteins Extracted from the Oomycete Pythium insidiosum and the Fungus Basidiobolus ranarum Compromises the Detection Specificity of Immunodiagnostic Assays for Pythiosis.	Journal of Fungi
2021	NA Extraction	Long-term effectiveness of bioaugmentation with rumen culture in continuous anaerobic digestion of food and vegetable wastes under feed composition fluctuations.	Bioresource Technology
2021	NA Extraction	Long-term monitoring of a thermal hydrolysis-anaerobic co-digestion plant treating high-strength organic wastes: Process performance and microbial community dynamics.	Bioresource technology
2020	Protein Synthesis	Automated Cell-Free Multiprotein Synthesis Facilitates the Identification of a Secretory, Oligopeptide Elicitor-Like, Immunoreactive Protein of the Oomycete Pythium insidiosum.	Msystems
2020	Protein Synthesis	Golgi-localized cyclophilin 21 proteins negatively regulate ABA signalling via the peptidyl prolyl isomerase activity during early seedling development.	Plant Molecular Biology
2020	NA Extraction	Magnetite-assisted in situ microbial oxidation of H2S to S0 during anaerobic digestion: a new potential for sulfide control.	Chemical Engineering Journal
2020	NA Extraction	Treatment of low-strength ammonia wastewater by single-stage partial nitritation and anammox using upflow dual-bed gel-carrier reactor (UDGR).	Bioresource Technology
2019	Protein Synthesis, Purification	The organization of tyrosine hydroxylase-immunopositive cells in the sparrow retina.	Neuroscience research
2019	NA Extraction	Nutrient removal and microalgal biomass production from different anaerobic digestion effluents with Chlorella species.	Scientific Reports

Year	Purpose	Title of paper	Publisher
2019	NA Extraction	Co-feeding spent coffee grounds in anaerobic food waste digesters: Effects of co-substrate and stabilization strategy.	Bioresource technology
2018	Protein Synthesis	Selectively modulating conformational states of USP7 catalytic domain for activation.	Structure
2018	Protein Synthesis	Receptor-mediated dimerization of JAK2 FERM domains is required for JAK2 activation.	Elife
2018	Protein Purification	Highly efficient genome editing by CRISPR-Cpf1 using CRISPR RNA with a uridinylate-rich 3′-overhang.	Nature communications
2018	NA Extraction	Anaerobic co-digestion of high-strength organic wastes pretreated by thermal hydrolysis.	Bioresource technology
2018	NA Extraction	A long-term study on the effect of magnetite supplementation in continuous anaerobic digestion of dairy effluent–Enhancement in process performance and stability.	Bioresource technology
2016	Protein Purification	Overexpression of OsCYP19-4 increases tolerance to cold stress and enhances grain yield in rice (Oryza sativa).	Journal of experimental botany
2016	Protein Synthesis	Removal of the mechanoprotective influence of the cytoskeleton reveals PIEZO1 is gated by bilayer tension.	Nature communications
2016	Protein Synthesis	The role of MscL amphipathic N terminus indicates a blueprint for bilayer-mediated gating of mechanosensitive channels.	Nature communications
2016	NA Extraction	Response of a continuous anaerobic digester to temperature transitions: A critical range for restructuring the microbial community structure and function.	Water research
2016	NA Extraction	Mild-temperature thermochemical pretreatment of green macroalgal biomass: effects on solubilization, methanation, and microbial community structure.	Bioresource technology

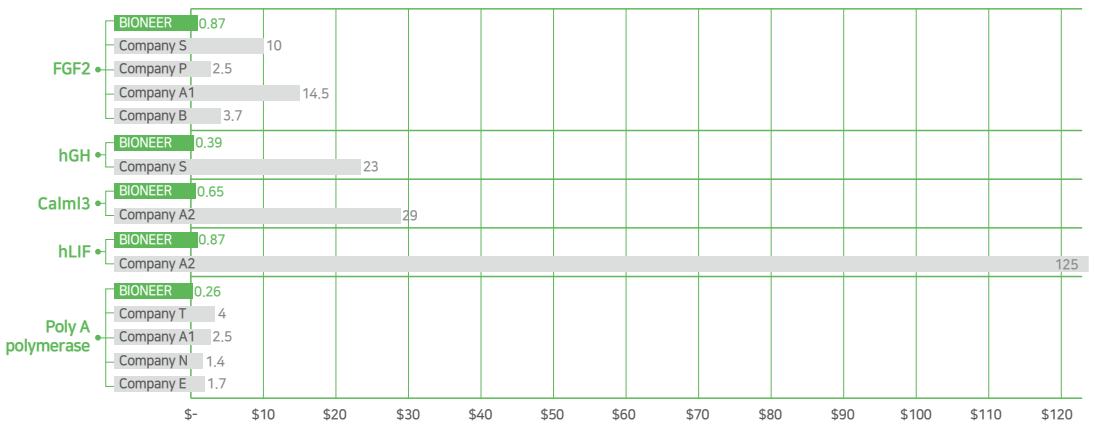
2.3. Commercial protein synthesis using *ExiProgen*™

The potential commercial value of *ExiProgen*™ is very high. It can synthesize proteins very easily with template DNA. There is no need to pay high prices for commercial proteins to use in laboratory, but you can synthesize your own using this instrument.

Comparison of Specification

Protein	Company	Volume	Price ($)	Unit cost ($/μg)
FGF2	BIONEER	30 μg	26	0.87
	Company S	25 μg	250	10
	Company P	50 μg	125	2.5
	Company A1	10 μg	145	14.5
	Company B	50 μg	188	3.7
hGH	BIONEER	66 μg	26	0.39
	Company S	10 μg	232	23
CalmI3	BIONEER	40 μg	26	0.65
	Company A2	10 μg	298	29
hLIF	BIONEER	30 μg	26	0.87
	Company A2	2 μg	250	125
Protein	**Company**	**Unit**	**Price ($)**	**Unit cost ($/Unit)**
Poly A polymerase	BIONEER	100 units	26	0.26
	Company T	100 units	425	4
	Company A1	80 units	200	2.5
	Company N	100 units	145	1.4
	Company E	50 units	85	1.7

Comparison of Unit Price of Commercial Products and In-house Proteins sythesized on *ExiProgen*™



Ordering Information

Cat. No.	Product Description
Instrument	
A-5041	*ExiProgen*™
Accessories	
A-5041-A	Accessories Set for *ExiProgen*™ (A2, 3, 4, 5, 6, 7, 10)
A-5041-A1	Multi Puncher (option)
A-5041-A2	Setup Tray
A-5041-A3	Disposable Tip Rack
A-5041-A4	Elution Tube Rack
A-5041-A5	Reaction Block (For protein synthesis)
A-5041-A6	Waste Tray
A-5041-A7	Hole Puncher (6-hole)
A-5041-A9	AC Adapter for *ExiProgen*™, *ExiPrep*™16 Dx
A-5041-A10	Contamination Shield
A-5041-A12	Protection Cover Separation Tool

* Accessories can be purchased after consultation with the Manufacture Team.

Please send an inquiry to the following address: sales@bioneer.co.kr

Part. II

Nucleic Acid Extraction

Introduction

BIONEER's *ExiProgen*™ is a fully automated instrument for high quality nucleic acid extraction from various biological sources such as blood, animal tissues, cultured cells, or plant tissues. The companion kits use magnetic silica bead (KR101555534B1) that have high DNA/RNA binding capacity and all reagents are pre-dispensed into a 'Pre-Loaded Buffer Cartridge System.' Furthermore, highly optimized protocols installed in *ExiProgen*™ for each sample ensure high efficiency and reproducibility of nucleic acid extraction.

Principles of Nucleic Acid (NA) Extraction

Sample Lysis

Chaotropic agents are widely used to facilitate nucleic acid purification using silica, which is known to cause an entropy effect in relation to structured water and collective salt bridge. BIONEER's lysis buffers contain guanidine hydrochloride or guanidine thiocyanate as chaotropic agents, which removes water molecules around cells, disrupting cell membrane and protein structure.

In the case of samples with cell walls such as gram-negative bacteria or yeast, it is necessary to decompose the cell wall through pretreatment such as using lysozyme that hydrolyzes cell wall.

Bead Binding

After cell lysis in which nucleic acid molecules are freely suspended, the silica magnetic bead encounters chaotropic agents and the water molecules on the surface of the bead gets substituted with cation molecules in the solution, making the bead positively polarized. Then the backbone of the nucleic acid, which is negatively charged due to the presence of anions, gets attached to the bead.

Magnetic Separation (washing and elution)

When nucleic acid is attached to magnetic beads, a magnetic field is used to attract the complex to a part of a tube wall, allowing only the unbound molecules to be washed with washing buffer. Through washing, the non-targeted proteins, cell debris, and excessive salts are washed away. Each wash buffer has been highly optimized for a specific biological sample in order to remove all potential contaminants, which can inhibit PCR. The captured nucleic acid is then eluted by PCR-compatible elution buffer, an aqueous solution with an optimal pH.

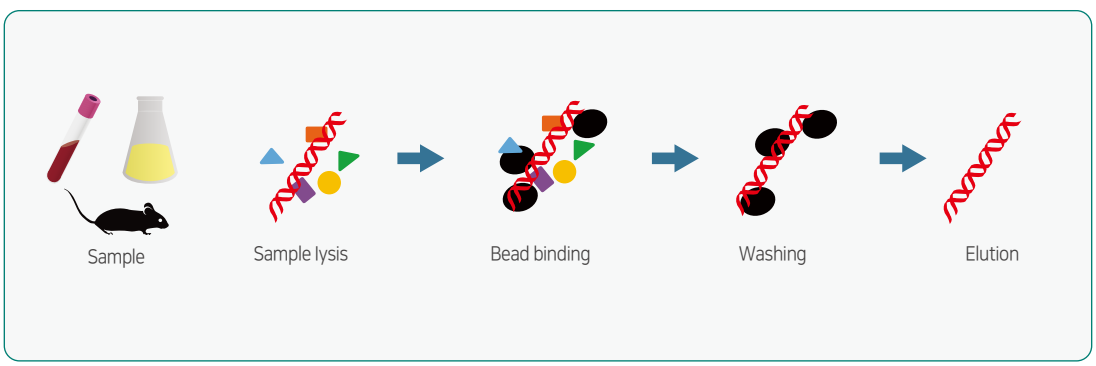


Figure 1. Nucleic Acid Extraction procedure using silica magnetic bead.

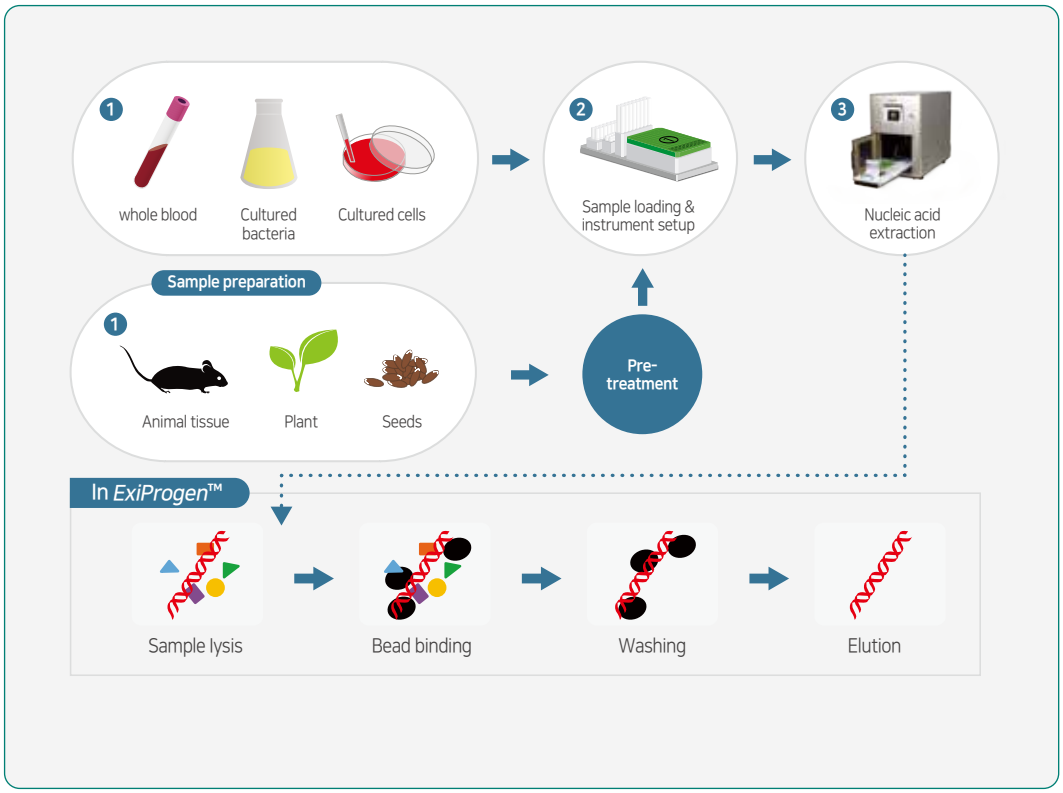
Chapter 1 - Automatic Nucleic Acid Extraction

Nucleic Acid Extraction Kit series, named as ***ExiPrep*™ Kit series**, extracts nucleic acids using patented silica magnetic beads (*AccuNanoBead*™).

- ✓ All reagents are pre-dispensed into a buffer cartridge system.
- ✓ Ensures high yield and purity with BIONEER's patented magnetic silica beads.
- ✓ Extract nucleic acids from up to 16 different samples simultaneously.
- ✓ Guarantees reproducible results with automated protocols.

In just three steps, the nucleic acid can be extracted in a fully-automated manner.

▶ Procedure

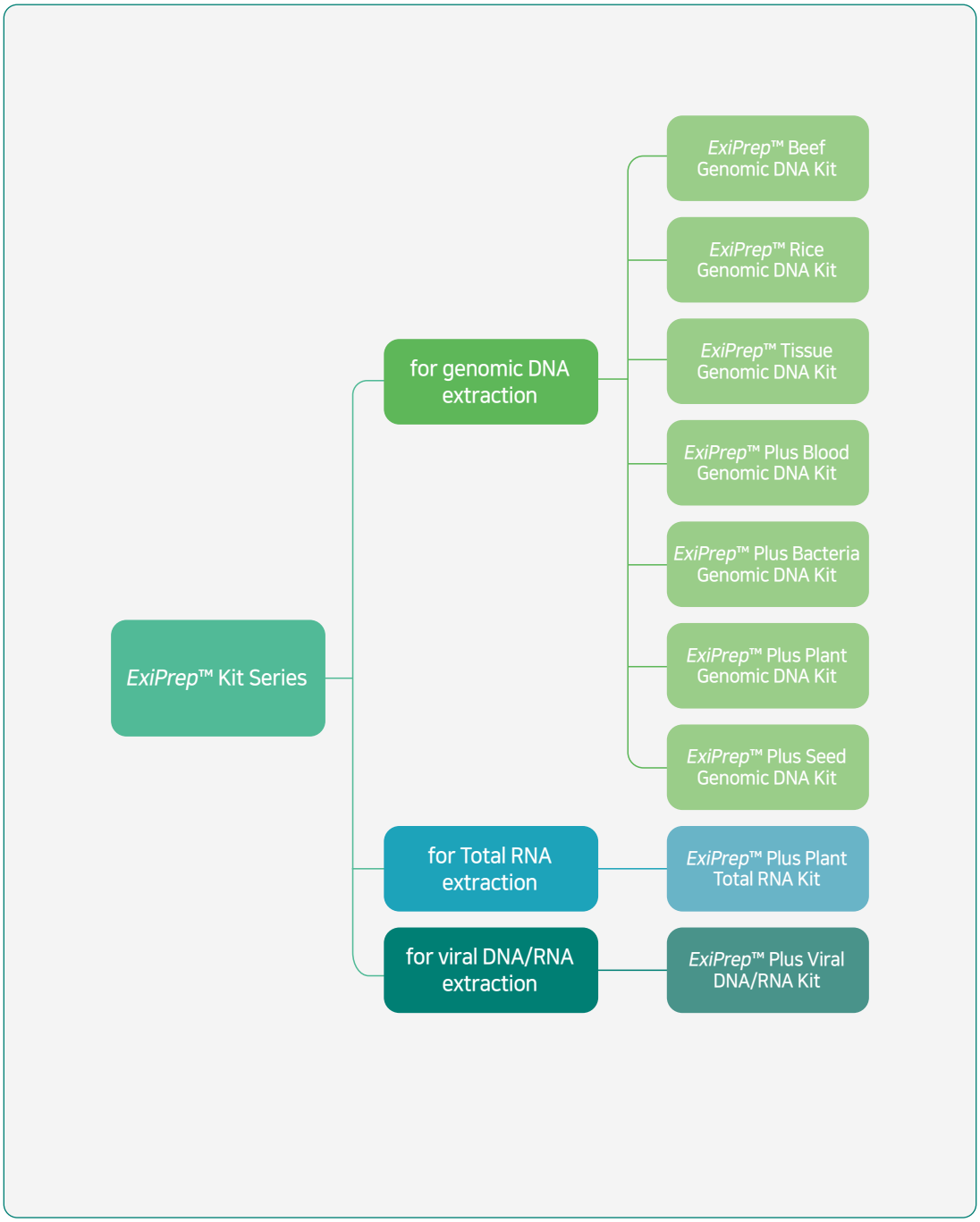


1. Prepare and pre-treat* the sample.
2. Load the sample and setup the instrument.
3. Select the correct Protocol No. and run the instrument.

* For high purity DNA extraction, the lysate should then be treated with Protease K or RNase A to remove protein or RNA. While for RNA extraction, the lysate is directly subjected to purification step, and must be handled with extensive care not to destroy nuclear membrane or be hydrolyzed by RNase, in order to extract high purity RNA.

Kit Selection Guide for NA Extraction

BIONEER's kits are individually optimized for specific biological samples. Product lines are divided into DNA extraction, RNA extraction and Viral DNA/RNA extraction kits. Each kit contains enzymes for nucleic acid extraction and pre-filled buffer cartridge, and other consumables like disposable filter tip, and elution tube – to extract nucleic acid from up to 16 samples simultaneously using *ExiProgen*™. Further, using our patented magnetic silica beads allows nucleic acid extraction with higher yield and purity.



1.1. Targeting Genomic DNA

ExiPrep™ Beef Genomic DNA Kit

[Cat. No. K-3200-CB]

This kit allows extraction of genomic DNA from beef to discriminate Korean beef, using automatic nucleic acid extraction instruments. Furthermore, Tissue Lysis buffer and Proteinase K provided in the kit can be used for sample preparation and pretreatment (methods described in the user manual), which helps effectively break down animal tissues.

Expected Yield	Up to 10 µg
Protocol No.	102
Running Time	1 hr 35 min
No. of samples	Up to 16

▶ Features and Benefits

- **Pre-filled buffer cartridge system**
  Dispensed enzymes and reagents essential for nucleic acid extraction.
- **Optimized lysis**
  Tissue Lysis Buffer and consumables (Disposable filter tip, Elution tube, etc.) included in the kit for various animal tissue lysis.
- **High purity, high quality and high-yield production of nucleic acid**
  Use of Silica Magnetic Particles developed and produced using BIONEER's proprietary technology.
- **High reproducibility**
  Reliable results through the use of automatic nucleic acid extraction instruments (*ExiPrep*™ 16 Plus, *ExiProgen*™).

▶ Experimental Data

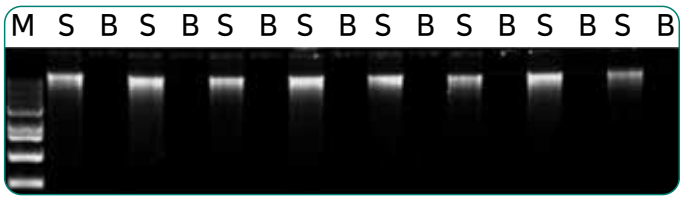


Figure 1. Electrophoresis results of genomic DNA extracted from bovine tissues.
Results of electrophoresis of genomic DNA extracted from bovine tissue samples (40 mg) on 1% agarose gel were shown above. To check the occurrence of cross-contamination during genomic DNA extraction, the bovine tissue samples were placed sparsely in a checkerboard pattern while the rest wells were filled with distilled water (D.W.). No signs of cross-contamination were found in the extracted genomic DNA, with the average yield of 10 µg and the mean purity ($A_{260/280}$) of more than or at least 1.8.
M: DNA Size Marker
S: Extraction with bovine tissue sample
B: Extraction with D.W. only

ExiPrep™ Rice Genomic DNA Kit

[Cat. No. K-3200-CR]

This kit allows rapid extraction of genomic DNA for a rice variety inspection using automatic nucleic acid extraction instrument. Furthermore, Rice Lysis buffer, Rice Binding buffer and Proteinase K provided in the kit can be used for sample preparation and pretreatment (methods described in the user manual), which helps effectively break down rice.

Expected Yield	1-2 μg
Protocol No.	106
Running Time	1 hr 5 min
No. of samples	Up to 16

▶ Features and Benefits

- **Pre-filled buffer cartridge system**
  Dispensed enzymes and reagents essential for nucleic acid extraction.
- **Optimized lysis**
  Rice Lysis Buffer and consumables (Disposable filter tip, Elution tube, etc.) included in the kit for shredded rice samples.
- **High purity, high quality and high-yield production of nucleic acid**
  Use of Silica Magnetic Particles developed and produced using BIONEER's proprietary technology.
- **High reproducibility**
  Reliable results through the use of automatic nucleic acid extraction instruments (*ExiPrep*™ 16 Plus, *ExiProgen*™).

▶ Experimental Data

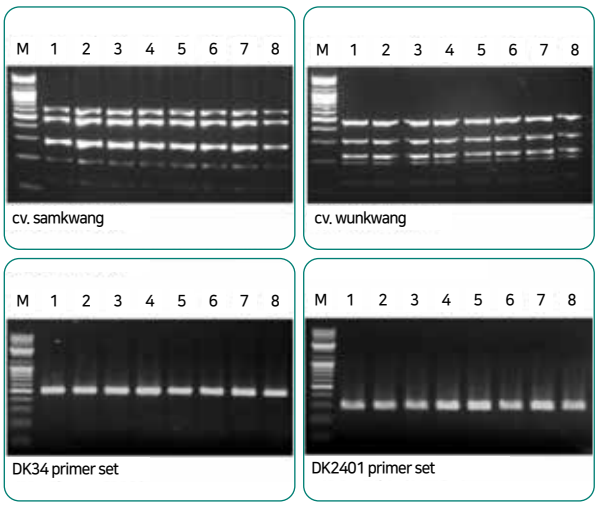


Figure 1. PCR results of genomic DNA extracted from rice sample.
Results of 1% agarose gel electrophoresis of PCR with extracted genomic DNA using each PCR primer sets.
M: DNA Size Marker
Lane 1-8: PCR product with extracted genomic DNA

1.1. Targeting Genomic DNA

ExiPrep™ Tissue Genomic DNA Kit

[Cat. No. K-3225]

This kit allows rapid extraction of highly pure genomic DNA with high yield from animal tissue samples, using automatic nucleic acid extraction instrument. Furthermore, Tissue Lysis buffer and Proteinase provided in the kit can be used for sample preparation and pretreatment, which helps effectively break down animal tissues.

Expected Yield	5-20 µg
Protocol No.	102 / 103
Running Time	1 hr 35 min
No. of samples	Up to 16

▶ Features and Benefits

- **Pre-filled buffer cartridge system**
  Dispensed enzymes and reagents essential for nucleic acid extraction.
- **Optimized lysis**
  Tissue Lysis Buffer and consumables (Disposable filter tip, Elution tube, etc.) included in the kit for various animal tissue lysis.
- **High purity, high quality and high-yield production of nucleic acid**
  Use of Silica Magnetic Particles developed and produced using BIONEER's proprietary technology.
- **High reproducibility**
  Reliable results through the use of automatic nucleic acid extraction instruments (*ExiPrep*™ 16 Plus, *ExiProgen*™).

▶ Experimental Data

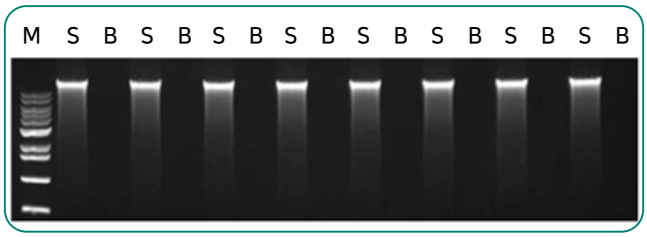


Figure 1. Agarose gel electrophoresis results of genomic DNA extracted from mouse tissue.

Results of electrophoresis of genomic DNA extracted from a mouse tail tip (~1 cm) on 1% agarose gel. To check the occurrence of cross-contamination during genomic DNA extraction, mouse tail-tip samples were placed sparsely in a checkerboard pattern while the rest wells were filled with distilled water (D.W.). No signs of cross-contamination were found in the extracted genomic DNA, with the average yield of 20 µg and the mean purity ($A_{260/280}$) of at least 1.8.

M: DNA Size Marker

S: Extraction with mouse tail-tip sample

B: Extraction with D.W. only

ExiPrep™ Plus Blood Genomic DNA Kit

[Cat. No. K-4211]

This kit provides a total solution for an accurate and rapid extraction of genomic DNA from whole blood, giving high purity end-product. The kit contains all buffers and consumables necessary for efficient and effective genomic DNA extraction.

Expected Yield	1-5 µg
Protocol No.	101
Running Time	1 hr 50 min
No. of samples	Up to 16

▶ Features and Benefits

- **Pre-filled buffer cartridge system**
  Dispensed enzymes and reagents essential for nucleic acid extraction.
- **Optimized lysis**
  Required consumables (Disposable filter tip, Elution tube, etc.) included in the kit for user-friendly extraction process.
- **High purity, high quality and high-yield production of nucleic acid**
  Use of Silica Magnetic Particles developed and produced using BIONEER's proprietary technology.
- **High reproducibility**
  Reliable results through the use of automatic nucleic acid extraction instruments (*ExiPrep*™ 16 Plus, *ExiProgen*™).

▶ Experimental Data

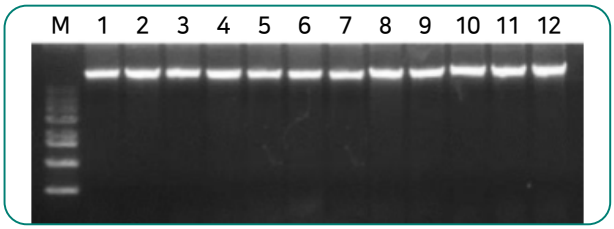


Figure 1. Genomic DNA from human whole blood (200 µl) using *ExiPrep*™ Plus Blood Genomic DNA Kit.

M: DNA Size Marker

Land 1-12: mean yield; 2.8 µg, mean purity; 1.8

1.1. Targeting Genomic DNA

ExiPrep™ Plus Bacteria Genomic DNA Kit

[Cat. No. K-4214]

This kit can be used to extract high-purity genomic DNA with high yield from Gram (-) bacteria, Gram (+) bacteria samples using automatic nucleic acid extraction instrument.

Expected Yield	5-15 μg
Protocol No.	Sample-dependent
Running Time	1 hr 52 min
No. of samples	Up to 16

▶ Features and Benefits

- **Pre-filled buffer cartridge system**
  Dispensed enzymes and reagents essential for nucleic acid extraction.
- **Optimized lysis**
  Resuspension Buffer and consumables (Disposable filter tip, Elution tube, etc.) included in the kit for re-suspension and lysis of Bacterial cell.
- **High purity, high quality and high-yield production of nucleic acid**
  Use of Silica Magnetic Particles developed and produced using BIONEER's proprietary technology.
- **High reproducibility**
  Reliable results through the use of automatic nucleic acid extraction instruments (*ExiPrep*™ 16 Plus, *ExiProgen*™).

▶ Experimental Data

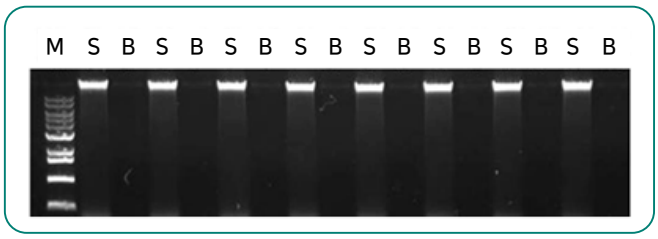


Figure 1. Results of experiment using *E. coli* cell (1×10^9 cells).

Results of 1% agarose gel electrophoresis of genomic DNA extracted from *E. coli* cell (1×10^9 cells). To observe cross-contamination that may occur during the extraction process, the *E. coli* cell samples were placed in a checkerboard pattern, and the rest of the wells were filled with D.W. And genomic DNA extraction was performed. As a results, no cross-contamination could be detected. The yields of extracted genomic DNA were found to be an average of 10 μg and the purity ($A_{260/280}$) averaged 1.8 or more.

M: DNA Size Marker

S: Extraction with *E. coli* cell sample

B: Extraction with D.W. only

ExiPrep™ Plus Plant Genomic DNA Kit

[Cat. No. K-4215]

This kit allows rapid extraction of highly-pure genomic DNA giving high-yield, from plant samples such as leaf tissue, seed, root, etc, using automatic nucleic acid extraction instrument. Furthermore, Plant Lysis Buffer and Proteinase K provided in the kit can be used for sample preparation and pretreatment (methods described in the user manual), which helps effectively break down plant tissues and remove impurities.

Expected Yield	0.2-5 μg
Protocol No.	104
Running Time	1 hr 20 min
No. of samples	Up to 16

▶ Features and Benefits

- **Pre-filled buffer cartridge system**
  Dispensed enzymes and reagents essential for nucleic acid extraction.
- **Optimized lysis**
  Plant Lysis Buffer and consumables (Disposable filter tip, Elution tube, etc.) included in the kit for various plant samples.
- **High purity, high quality and high-yield production of nucleic acid**
  Use of Silica Magnetic Particles developed and produced using BIONEER's proprietary technology.
- **High reproducibility**
  Reliable results through the use of automatic nucleic acid extraction instruments (*ExiPrep*™ 16 Plus, *ExiProgen*™).

▶ Experimental Data

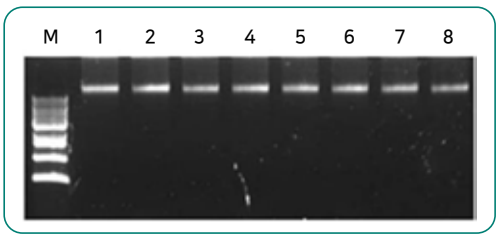


Figure 1. Electrophoresis results of genomic DNA extracted from various plants.

Results of electrophoresis of genomic DNA extracted from various samples done on 1% agarose gel.
It can be seen that the DNA was extracted intact without any degradation, with its average yield of 1-3 μg and its purity ($A_{260/280}$) of 1.8 or more.
M: DNA Size Marker
Lane 1-8: Extracted genomic DNA

1.1. Targeting Genomic DNA

ExiPrep™ Plus Seed Genomic DNA Kit

[Cat. No. K-4217]

This kit allows rapid extraction of highly-pure genomic DNA giving high-yield, from plant samples such as tissue, seed, root, etc, using automatic nucleic acid extraction instrument. Furthermore, Seed Lysis Buffer and Proteinase K provided in the kit can be used for sample preparation and pretreatment (methods described in the user manual), which helps effectively break down plant tissues and remove impurities.

Expected Yield	0.25-5 μg
Protocol No.	105
Running Time	1 hr 17 min
No. of samples	Up to 16

▶ Features and Benefits

- **Pre-filled buffer cartridge system**
  Dispensed enzymes and reagents essential for nucleic acid extraction.
- **Optimized lysis**
  Plant Lysis Buffer and consumables (Disposable filter tip, Elution tube, etc.) included in the kit for various plant samples.
- **High purity, high quality and high-yield production of nucleic acid**
  Use of Silica Magnetic Particles developed and produced using BIONEER's proprietary technology.
- **High reproducibility**
  Reliable results through the use of automatic nucleic acid extraction instruments (*ExiPrep*™ 16 Plus, *ExiProgen*™).

▶ Experimental Data

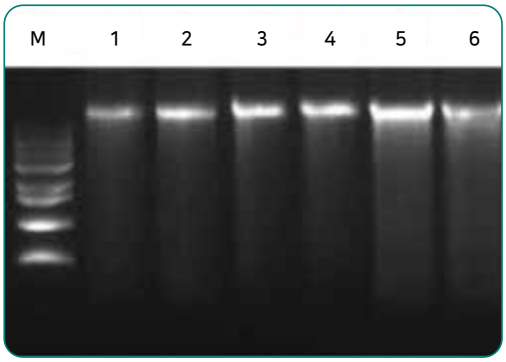


Figure 1. Comparison of genomic DNA extracted from Callistephus chinensis seed (50 mg) using *ExiPrep*™ Plus Seed Genomic DNA Kit [Cat. No. K-4217, automatic], *ExiPrep*™ Plus Plant Genomic DNA Kit [Cat. No. K-4215, automatic], and *MagListo*™ 5M Plant Genomic DNA Extraction Kit [Cat. No. K-3605, manual].

M: DNA Size Marker
Lane 1-2: *ExiPrep*™ Plus Seed Genomic DNA Kit;
mean yield: 4.4 μg, mean purity: 1.58
Lane 3-4: *ExiPrep*™ Plus Plant Genomic DNA Kit;
mean yield: 4.7 μg, mean purity: 1.66
Lane 5-6: *MagListo*™ 5M Plant Genomic DNA Extraction Kit;
mean yield: 5.2 μg, mean purity: 1.61

1.2. Targeting Total RNA

ExiPrep™ Plus Plant total RNA Kit

[Cat. No. K-4244]

This kit allows rapid extraction of highly-pure genomic DNA giving high-yield, from various plant samples such as leaf tissue, seed, root, flower, etc., using automatic nucleic acid extraction instrument. Furthermore, Plant Lysis Buffer and β-Mercaptoethanol provided in the kit can be used for sample preparation while suppressing RNA degradation through RNase.

Expected Yield	5-15 μg
Protocol No.	204 / 205
Running Time	1 hr 20 min
No. of samples	Up to 16

▶ Features and Benefits

- **Pre-filled buffer cartridge system**
  Dispensed enzymes and reagents essential for nucleic acid extraction.
- **Optimized lysis**
  Plant Lysis Buffer and consumables (Disposable filter tip, Elution tube, etc.) included in the kit for various plant samples.
- **High purity, high quality and high-yield production of nucleic acid**
  Use of Silica Magnetic Particles developed and produced using BIONEER's proprietary technology.
- **High reproducibility**
  Reliable results through the use of automatic nucleic acid extraction instruments (*ExiPrep*™ 16 Plus, *ExiProgen*™).

▶ Experimental Data

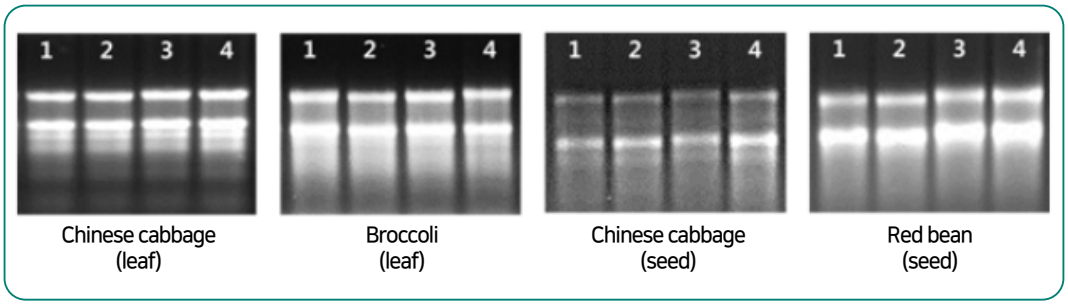


Figure 1. Results from analyzing total RNA extracted from various plant samples.

Results of agarose gel electrophoresis of total RNA extracted from various plant samples are shown above. Each subunit of rRNA can be clearly seen, implying the intact extraction of RNA during the extraction process without any degradation.

1.3. Targeting Viral DNA/RNA

ExiPrep™ Plus Viral DNA/RNA Kit

[Cat. No. K-4271]

This kit allows rapid extraction of highly-pure viral DNA/RNA giving high-yield, from various samples such as serum, plasma, saliva, etc., using automatic nucleic acid extraction instrument.

Protocol No.	Sample-dependent
Running Time	1 hr 43 min
No. of samples	Up to 16

▶ Features and Benefits

- **Pre-filled buffer cartridge system**
  Dispensed enzymes and reagents essential for nucleic acid extraction.
- **Optimized lysis**
  Pre-treatment methods and consumables (Disposable filter tip, Elution tube, etc.) included in the kit for various sample lysis.
- **High purity, high quality and high-yield production of nucleic acid**
  Use of Silica Magnetic Particles developed and produced using BIONEER's proprietary technology.
- **High reproducibility**
  Reliable results through the use of automatic nucleic acid extraction instruments (*ExiPrep*™ 16 Plus, *ExiProgen*™).

▶ Experimental Data

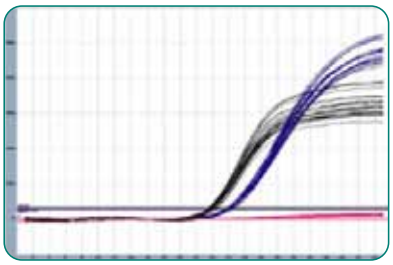


Figure 1. Viral DNA Extraction using *ExiPrep*™ Plus Viral DNA/RNA Kit from HBV serum.

Results gained from real-time PCR using *AccuPower*® HBV Quantitative PCR Kit [Cat. No. HBV-1111] and *Exicycler*™ 96 Real-Time Quantitative Thermal Block [Cat. No. A-2060] after extracting HBV viral DNA from serum samples.

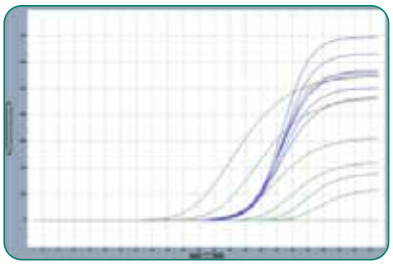


Figure 2. Viral RNA Extraction using *ExiPrep*™ Plus Viral DNA/RNA Kit from HCV serum.

Results gained from real-time RT-PCR using *AccuPower*® HCV Quantitative PCR Kit [Cat. No. HCV-1111] and *Exicycler*™ 96 Real-Time Quantitative Thermal Block [Cat. No. A-2060] after extracting HCV viral RNA from serum samples.

Summary of Nucleic Acid Extraction Kit Series

Kit type & Application

Product for Genomic DNA Extraction	Cat. No.	Sample Source
ExiPrep™ Beef Genomic DNA Kit	K-3200-CB	Animal tissue
ExiPrep™ Rice Genomic DNA Kit	K-3200-CR	Rice
ExiPrep™ Tissue Genomic DNA Kit	K-3225	Animal tissue FFPE tissue
ExiPrep™ Plus Blood Genomic DNA Kit	K-4211	Whole Blood
ExiPrep™ Plus Bacteria Genomic DNA Kit	K-4214	Gram (+/-) bacteria Cultured cells (HeLa cells) Yeast, etc.
ExiPrep™ Plus Plant Genomic DNA Kit	K-4215	Plant tissue
ExiPrep™ Plus Seed Genomic DNA Kit	K-4217	Plant seed
Product for Total RNA Extraction	**Cat. No.**	**Sample Source**
ExiPrep™ Plus Plant total RNA Kit	K-4244	Plant tissue Plant seed
Product for Viral DNA/RNA Extraction	**Cat. No.**	**Sample Source**
ExiPrep™ Plus Viral DNA/RNA Kit	K-4271	Serum Plasma Urine, etc.

Protocol Number & Protocol Name

Product Description	Protocol No.	Sample Source
ExiPrep™ Beef Genomic DNA Kit	102	Genomic DNA: Animal tissue
ExiPrep™ Rice Genomic DNA Kit	106	Genomic DNA: Rice
ExiPrep™ Tissue Genomic DNA Kit	102 103	Genomic DNA: Animal tissue Genomic DNA: FFPE tissue
ExiPrep™ Plus Blood Genomic DNA Kit	101	Genomic DNA: Whole Blood
ExiPrep™ Plus Bacteria Genomic DNA Kit	Sample-dependent	Sample-dependent
ExiPrep™ Plus Plant Genomic DNA Kit	104	Genomic DNA: Plant tissue
ExiPrep™ Plus Seed Genomic DNA Kit	105	Genomic DNA: Plant seed
ExiPrep™ Plus Plant total RNA Kit	204 205	Total RNA: Plant tissue Total RNA: Plant seed
ExiPrep™ Plus Viral DNA/RNA Kit	Sample-dependent	Sample-dependent

* For protocol numbers that are sample-dependent, refer to the User Guide of the instrument *ExiProgen*™.

Troubleshooting Guide

Please refer to the list below for some of the commonly asked questions and solutions if you encounter problems while using nucleic acid extraction products. The solutions listed below are only general suggestions and might not address all problems.

1. Low yield of Genomic DNA

Cause	Solution
Added too much or too little sample	The yield is dependent on the sample type and the starting amount. Too much or too little sample will decrease yields.
Incomplete lysis of sample	Make sure the lysate is completely cleared via centrifugation. Incomplete lysis and clearing decreases the yield and purity.
Buffer cartridge ① not agitated/ suspended	Incomplete suspension of magnetic beads may decrease the yield and purity.

2. Co-eluted magnetic particle

Explanation
Sometimes magnetic particles are carried-over with your Genomic DNA after elution. Carryover of magnetic particles in the eluate will not affect the performance of the genomic DNA in downstream applications. Furthermore, magnetic particle cannot bind to Genomic DNA in elution buffer, though it may affect readings on a spectrophotometer. Magnetic particles that are carried over can be easily separated by centrifugation for 1 min at 13,000 rpm in a microcentrifuge.

Ordering Information

Cat. No.	Product Description
Genomic DNA Extraction	
K-3200-CB	*ExiPrep*™ Beef Genomic DNA Kit
K-3200-CR	*ExiPrep*™ Rice Genomic DNA Kit
K-3225	*ExiPrep*™ Tissue Genomic DNA Kit
K-4211	*ExiPrep*™ Plus Blood Genomic DNA Kit
K-4214	*ExiPrep*™ Plus Bacteria Genomic DNA Kit
K-4215	*ExiPrep*™ Plus Plant Genomic DNA Kit
K-4217	*ExiPrep*™ Plus Seed Genomic DNA Kit
Total RNA Extraction	
K-4244	*ExiPrep*™ Plus Plant total RNA Kit
Viral DNA/RNA Extraction	
K-4271	*ExiPrep*™ Plus Viral DNA/RNA Kit

Related Products

Cat. No.	Product Description
PCR Premix Kit Series	
K-2012	*AccuPower*® PCR PreMix
K-2601	*AccuPower*® *Taq* PCR PreMix
K-2012-1	*AccuPower*® PCR PreMix (with UDG)
K-5050	*AccuPower*® *HotStart* PCR PreMix
K-2611	*AccuPower*® *PyroHotStart* PCR PreMix
K-5050-1	*AccuPower*® *HotStart* PCR PreMix (with UDG)
K-2022	*AccuPower*® *Pfu* PCR PreMix
K-2631	*AccuPower*® *ProFi Taq* PCR PreMix
K-2111	*AccuPower*® Multiplex PCR PreMix
K-2115	*AccuPower*® Gold Multiplex PCR PreMix
Instrument	
A-2041	*AllInOneCycler*™ PCR system
A-2060	*Exicycler*™ 96
A-5030	*ExiPrep*™16 Plus
A-5250	*ExiPrep*™96 Lite
A-7040	*ExiSpin*™
A-7020	*Agaro-Power*™ System
Ladder	
D-1030	*AccuLadder*™ 100 bp DNA Ladder
D-1040	*AccuLadder*™ 1kb DNA Ladder
D-2030	*AccuLadder*™ 3-color Prestained Protein Size Marker (Broad)
Buffer	
C-9100	Agarose
C-9002	5X TBE
C-9004	50X TAE
C-9027	10% Sodium Dodecyl Sulfate (SDS), 500 ml
C-9029	6X Agarose Gel Loading Buffer

Part. III

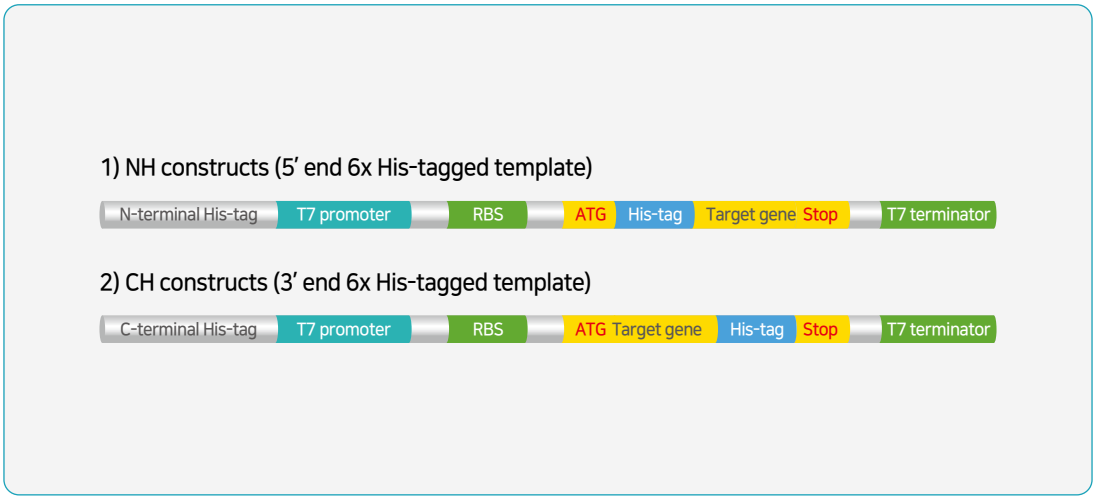
DNA Template Preparation

Introduction

Using BIONEER's kits, template DNA can be prepared in an easier and faster manner. This part of the Guide Book tells the criteria and the tips when preparing template DNA, as well as how to prepare template DNA at different cases.

Criteria: Template DNA Structure for *ExiProgen*™

To use *ExiProgen*™ for protein synthesis, it requires certain sequence of template DNA for cell-free protein expression. The template DNA must contain sequences of a "T7 promoter – Ribosome Binding Site (RBS) – target gene – T7 terminator". The target gene must also contain a start codon (ATG), 6x His-tag (for protein purification) at its 5′ or 3′ end, and a stop codon (TAA, TAG, TGA) at the other end. The schematic below visualizes the structure.



Tip 1: Recommended DNA purity for greater yield

BIONEER recommends use of highly pure DNA having $A_{260/280}$: 1.7-2.0 and $A_{260/230}$: >1.5, which is based on the observation that DNA purity affects the protein yield (Part III, Chapter 3). *AccuPrep*® PCR/Gel Purification Kit [Cat. No. K-3038] can be used to obtain high quality DNA.

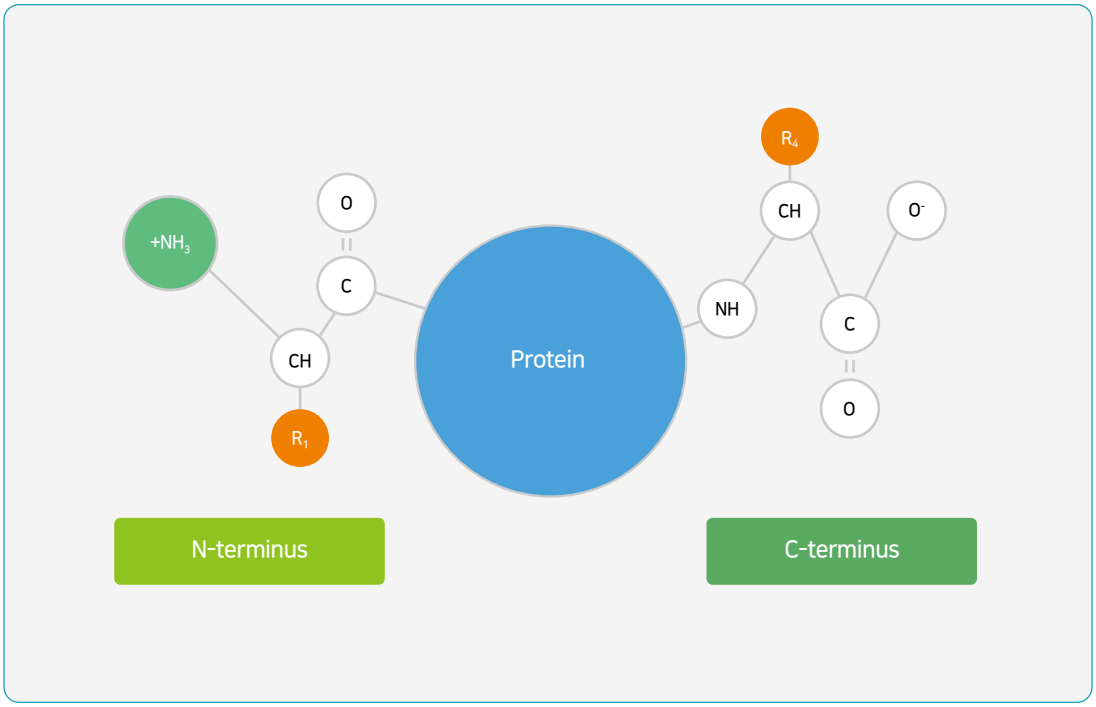
Tip 2: Codon optimization for *E. coli* codon

Codon usage is another factor affecting protein yield. BIONEER offers several services for Codon Optimization. AccuGeneBlock Synthesis Service provides *E. coli* codon as a PCR product, which is in linear form. On the other hand, Gene Synthesis Service provides *E. coli* codon as an *in vitro* translation vector, which is in plasmid form.

Further, Codon Optimization Service can also be done upon request, where the codon sequence is optimized for *E. coli* extract.

* Note: Both Codon Optimization & AccuGeneBlock Synthesis services can be ordered from the ordering page of [Gene Synthesis Custom Order].

Tip 3: Effect of His-tag position on protein



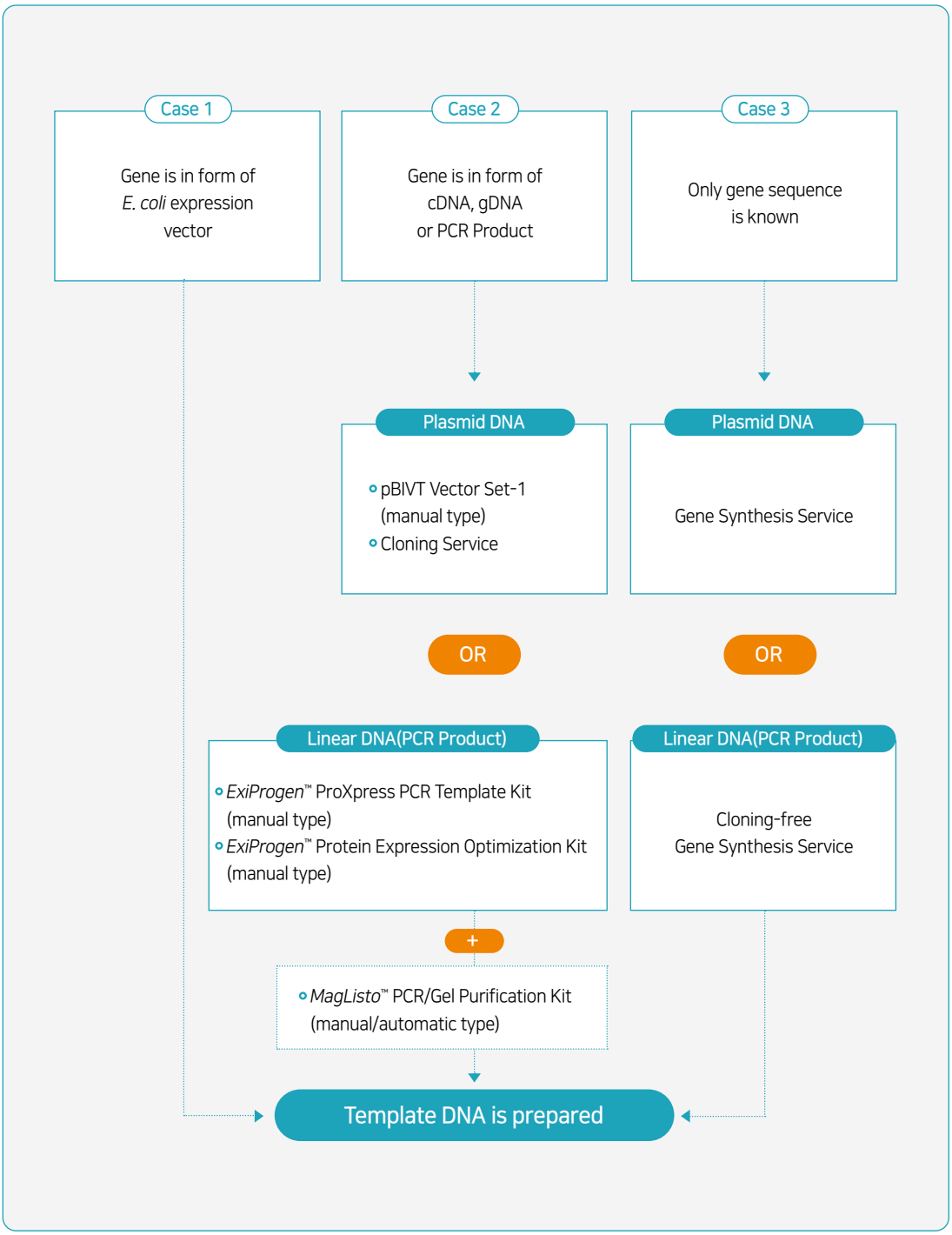
A 6x His-tag is required in the purification step when expressing protein using *ExiProgen*™. His-tag can be located either at the N- or C-terminus of the target protein. In general, it is known that the existence of 6x His-tag has no significant effect on the structure of a native protein (Carson et al., 2007). But the possibility exists that His-tag gets incorporated into the tertiary structure of protein making it inaccessible to Ni column, which makes it harder to purify the protein on Ni-NTA beads. In such cases, the location of His-tag can be changed from N- to C-terminus, or vice versa.

The effect of His-tag position on enzymatic activity was also suggested where N-terminal His-tag did not affect the activity of an enzyme from *Solanum dulcamara*, while C-terminal tagged form was functionally impaired (Freydank et al., 2008).

Chapter 1 - Preparation of Template DNA

1.1 Selection Guide for Template Preparation

Now, try to find which case you fall into, and follow the suggestions for each case.



Case I: Gene is in form of *E. coli* expression vector

When a target gene sequence is already included in a T7 expression vector sequence (pET, pK7, pIVEX, etc.) with 6x His-tag sequence attached, the gene can straight away be used as a template DNA for *ExiProgen*™.

Case II: Gene is in form of cDNA, gDNA or PCR product

When template DNA is cDNA, genomic DNA, or PCR product, it would be prepared in form of plasmid DNA and linear DNA (PCR product). BIONEER offers pBIVT Vector Set-1 [Cat. No. K-7350] and cloning service for plasmid DNA. If user has T7 expression vectors (pET, pK7, pIVEX etc.), template DNA would be inserted into the vector. At this time, gene sequence for protein expression must have 6x His-tag sequence.

with 6x His-tag sequence. For Linear DNA (PCR product), it can be prepared through two-step PCR using *ExiProgen*™ ProXpress PCR Template Kit [Cat. No. K-7400, K-7401].

When the protein of interest is hard to express, *ExiProgen*™ Protein Expression Optimization Kit [Cat. No. K-7410] would be the best option for that. *ExiProgen*™ Protein Expression Optimization Kit transforms template DNA into linear DNA with inserting tag sequence that improves protein expression. A total of six types of tag sequences can be attached, so user can select tags for protein expression level optimization.

Case III: Only gene seqeunce is known

When user only has gene sequence of target protein (ex. NCBI access's Gene Synthesis Service [Gene Synthesis, Gene Synthesis & Cloning, and AccuGeneBlock]. Using Gene Synthesis service of plasmid DNA, it is available to codon optimization and insert into T7 expression vector (pBT7-N-His vector, pBT7-C-His vector). In case of linear DNA (PCR product), Bioneer offers AccuGeneBlock service, and at this time, it has to order with T7 promoter, RBS, 6x His-tag and T7 terminator sequence.

1.2 Quick Synthesis of Template DNA using PCR

ExiProgen™ ProXpress PCR Template Kit

[Cat. No. K-7400, K-7401]

This manual-type kit is used to prepare template DNA by running PCR without undergoing cloning process. The template DNA prepared is in linear form. The pre-filled plate also provides all the required reagents except for the 1st primer set, which is needed to amplify the target gene. The amplified template DNA can then be used to produce a target protein on manual procedure or on *ExiProgen*™.

▶ Features and Benefits

- **Rapid process**
  Acquisition of template DNA is done through PCR only but cloning is not required.
- **User-friendly**
  All the reagents required for template DNA production is included except the primer sets [Cat. No. N-8229, 8230], which is used to amplify primary PCR target.
- **Minimized PCR error**
  Includes high-fidelity premix having high accuracy and precision, *AccuPower® ProFi Taq* PCR PreMix, to minimize the error rate during PCR.
- **Flexibility**
  Preparation of template DNA with His-tag at desired positions with various sizes.

▶ Procedure and Principle

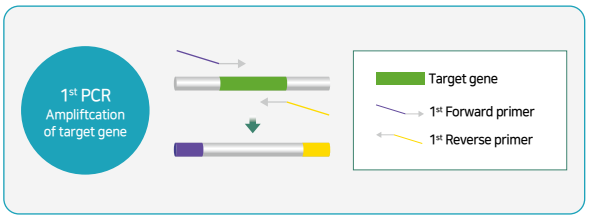
A) Order primers for protein synthesis

- To order N-terminal 6x histidine tag, select [Cat. No. N-8229].
- To order C-terminal 6x histidine tag, select [Cat. No. N-8230].
- And enter the desired sequences in the ordering site (eng.bioneer.com).
- Get the finalized first primer sets.

Forwerd Primer	5'-XXXXXXXXXXXXXXXXXXXXXXGAGAAAAAAATCACTGGA-3'(39-mer) Overlapping on upstream cassette
Reverse Primer	5'-XXXXXXXXXXXXXXXXXXXXXXCGCCCCGCCCTGCCACTC-3'(39-mer) Overlapping on downstream cassette

B) 1st PCR:

Primary target gene amplification using the ordered First primer F/R sets [Cat. No. N-8229, 8230] and *AccuPower® ProFi Taq* PCR Premix included in the kit.



C) 2nd Overlapping PCR:

Template preparation for protein synthesis using the first PCR product as its template DNA, with primer sets and cassette sets included in the kit.

D) Receive synthesized template DNA structures with *ExiProgen*™ ProXpress PCR Template Kit.

▶ Experimental Data

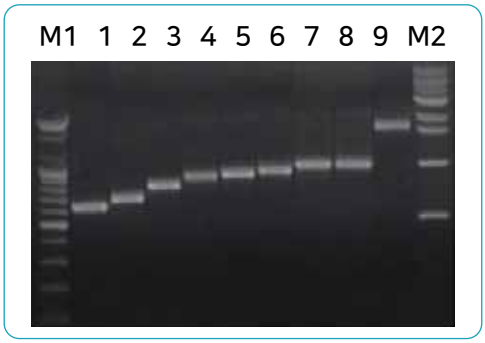


Figure 1. Synthesis of linear template DNA with *ExiProgen*™ ProXpress PCR Template Kit.
Each sample is loaded 100 ng on 1% TBE agarose gel.

M1: 100 bp DNA Ladder (Cat. No. D-1030)
Lane 1: SAV (0.6 kb Template – pT)
Lane 2: RNase H (0.7 kb, Template – BL21 (DE3) gDNA)
Lane 3: hGH (0.8 kb, Template – pT)
Lane 4: CAT (0.9 kb, Template - pBIVT)
Lane 5: UDG (0.95 kb, Template - BL21 (DE3) gDNA)
Lane 6: AcGFP (0.97 kb, Template – pBIVT)
Lane 7: EVO (1 kb, Template –pT)
Lane 8: RFP (1 kb, Template – pIVEX)
Lane 9: Poly A polymerase (1.6 kb, Template – pET15b)
M2: 1kb DNA Ladder (Cat. No. D-1040)

HIT!

ExiProgen™ Protein Expression Optimization Kit [Cat. No. K-7410]

This manual-type kit is used to generate linear template DNA that contains six different types of tag sequences, which can be used to screen various tags for maximizing the target protein expression. This enables expressing proteins that were once difficult to express using the standard *ExiProgen*™ protein synthesis instrument.

Furthermore, each of the template DNA contains TEV cleavage sites, allowing removal of tags after the protein synthesis if needed.

▶ Features and Benefits

- **Rapid process**
  Acquisition of template DNA is done through PCR only but cloning is not required.
- **Convenience**
  Contains all the components essential for generating maximum of 30 different types of template DNA (1st primer set must be separately ordered for first PCR).
- **Minimized PCR error**
  Includes high-fidelity premix having high accuracy and precision, *AccuPower® ProFi Taq* PCR PreMix, to minimize the error rate during PCR.

▶ Procedure and Principle

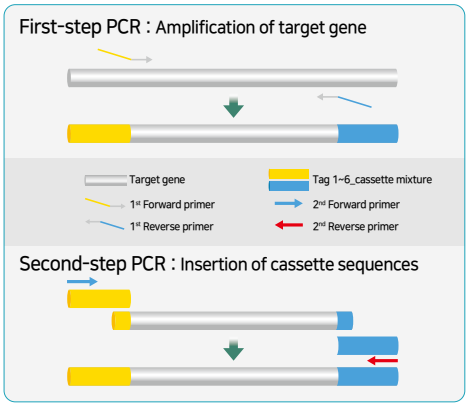
A) 1st PCR Primer Design

- Design the gene-specific primer as shown below.

1st Forward Primer	5'-GAGCTCGAAAACTTATATTTTCAGGGC+21-mer from the garget gene's 5' end-3' (48-mer)
1st Reverse Primer	5'-GGGCTTTGTTAGCAGCCGGTCGACCTA+21-mer from the garget gene's 3' end in reverse complementary sequence-3' (48-mer)

- Prepare designed primers through BIONEER's DNA Oligo Service.

B) Template DNA Synthesis



1st PCR:

Amplify the gene of interest by using the extended primer can be prepared with our customized service containing parts of cassettes.

2nd PCR:

Produce the template DNA with an overlapping PCR by using the 1st PCR products and the cassette that you wish to use.

▶ Experimental Data

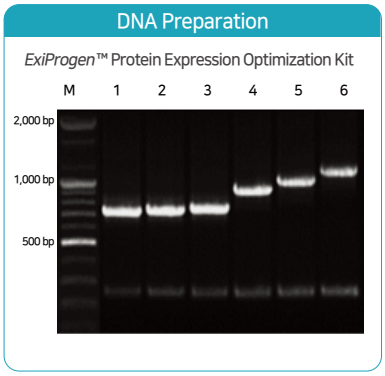


Figure 1. Template DNA construction using the control DNA.

Each linear DNA was generated by using *ExiProgen*™ Protein Expression Optimization Kit and 2nd PCR product samples are loaded on 1% agarose gel. The length of the control DNA is 462 bp.

M: DNA Ladder

Lane 1-6: Tag 1-6 inserted samples

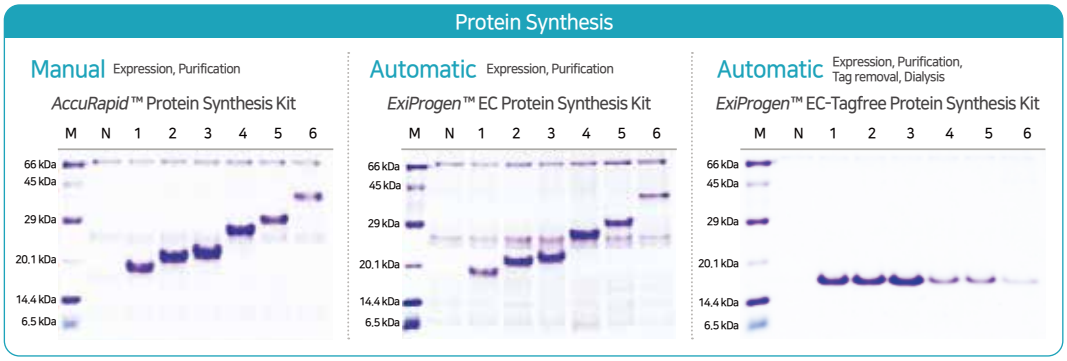


Figure 2. SDS-PAGE result of synthesized protein of the control DNA.

The linear DNA produced by using *ExiProgen*™ Protein Expression Optimization Kit was used as template DNA for protein synthesis with our various protein synthesis kits. The 22.5 µl of the purified proteins were loaded on 12% SDS-PAGE gel synthesized using 1 µg of DNA. The molecular weight of the control protein is 18 kDa.

M: Protein Size Marker

N: Non-tagged samples

Lane 1-6: Tag 1-6 inserted samples

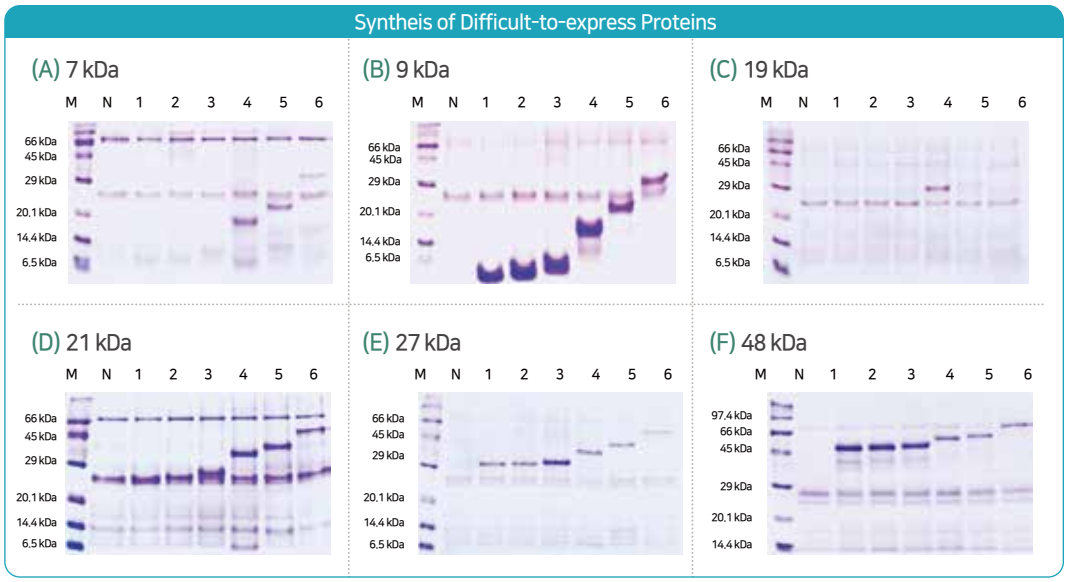


Figure 3. SDS-PAGE result of synthesized proteins of various sizes of difficult-to-express proteins.

Difficult-to-express proteins were synthesized using the template DNAs generated by the *ExiProgen*™ Protein Expression Optimization Kit and *ExiProgen*™ EC Protein Synthesis Kit.

M: Protein Size Marker

N: Non-tagged samples

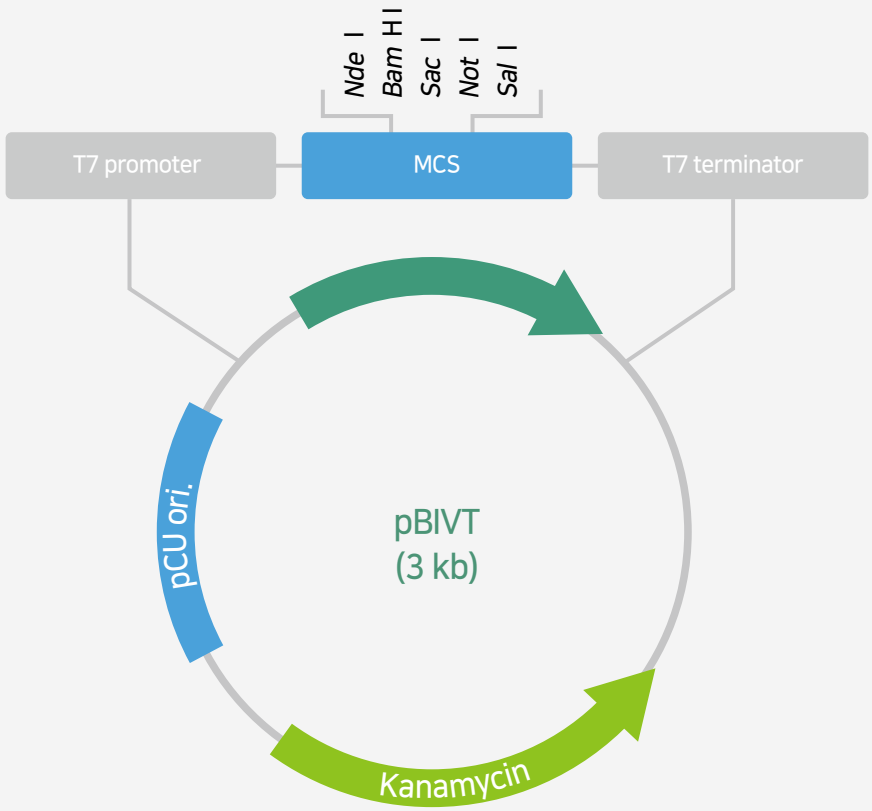
Lane 1-6: Tag 1-6 inserted sample

1.3. Cloning in pBIVT Vectors

pBIVT Vector Set-1 (each 5 μg)

[Cat. No. K-7350]

pBIVT Vector Set-1 (pBIVT-1, pBIVT-2) is a vector for cell-free protein expression kits, also compatible with all *ExiProgen*™ kit series. The vector can be used as a template DNA for cell-free protein expression. The gene of interest can be fused to His-tag at either its 5′ or 3′ end using this vector set. The structure of the vector is as shown below.



• pBIVT-1's MCS (Multiple Cloning Site) sequences

ATG	CATATG	CACCACCACCACCACCAC	GGATCC	GAGCTC	AAGCTT	GCGGCCGC	A	TAG	GTCGAC
Start codon	Nde I	6X His-tag		BamH I	Sac I		Not I	Stop codon	Sal I

• pBIVT-2's MCS (Multiple Cloning Site) sequences

ATG	CATATG	GGATCC	GAGCTC	AAGCTT	GCGGCCGC	A	CACCACCACCACCACCAC	TAG	GTCGAC
Start codon	Nde I	BamH I	Sac I		Not I		6X His-tag	Stop codon	Sal I

1.4. Purification of PCR Products

MagListo™ PCR/Gel Purification Kit

[Cat. No. K-3627G]

This kit consists of magnetic nanobeads and various buffers for PCR/Gel purification. The kit is designed to purify fragment DNAs of PCR products or other DNA products from different enzymatic reactions (including that of restriction enzyme, A-tailing, labeling, etc.) and extract fragment DNA from a TAE or TBE agarose gel. Purification can be done both manually or automatically, respectively using *MagListo*™ Magnetic Separation Rack or the instrument *ExiProgen*™ with *ExiProgen*™ Consumable SET.

Experiment	Gel purification	PCR purification
Protocol No.	351	352

* *MagListo*™ Kit series are manual type, and not automatic type. However, this kit can be automated in combination with the *ExiProgen*™ Consumable SET [Cat. No. KA-3001].

▶ Features and Benefits

- **Reduced experimental time**
  Magnetic nanobeads allow isolation from PCR products and various enzymatic reactants within only 5 minutes.
- **Wide range of uses**
  Can be used on low-melting agarose gels, TAE, and TBE agarose gel.
- **One kit, two uses**
  Both PCR and gel purification can be done using a single kit.
- **Manual Use**
  To use manually, *MagListo*™ Magnetic Separation Rack (not included) is needed, which allows a fast and easy purification with the use of magnetic beads. Hence, it shows greater efficiency and purity than when using a centrifuge. It helps remove various contaminants in the solution such as dimer, salts, dNTPs, enzymes, mineral oil, dye, detergent, etc. leaving only the required fragment DNA behind.
- **Automatic Use**
  For an automated purification, the instrument *ExiProgen*™ and *ExiProgen*™ Consumable SET [Cat. No. KA-3001], are needed. The procedure is very straight-forward and simple, as listed below.

  1) Dispense the reagents (components of the kit) into responsible positions in the cartridges (from *ExiProgen*™ Consumable SET)
  2) Install the cartridges and accessories onto the instrument
  3) Select a proper protocol for [PCR purification] / [Gel purification], and run the instrument

Ordering Information

Template Preparation Kits

Cat. No.	Product Description
K-7400~1	*ExiProgen*™ ProXpress PCR Template Kit (16/32 reactions)
K-7410	*ExiProgen*™ Protein Expression Optimization Kit
K-7350	*ExiProgen*™ pBIVT Vector Set-1 (each 5 μg)

Related Products

Cat. No.	Product Description
Buffer	
C-9100	Agarose, 100 g
C-9002	5X TBE, 1 gal
C-9004	50X TAE, 500 ml
C-9029	6X Agarose Gel Loading Buffer, 2 ml
C-9027	10% Sodium Dodecyl Sulfate (SDS), 500 ml
Instrument	
A-2041	*AllInOneCycler*™ PCR system
A-7040	*ExiSpin*™
A-7020	*Agaro-Power*™ System
DNA Ligase	
E-3061	T4 DNA Ligase
K-7103	*AccuPower*® Ligation PreMix
Ladder	
D-1030	*AccuLadder*™ 100 bp DNA Ladder
D-1040	*AccuLadder*™ 1kb DNA Ladder
D-2030	*AccuLadder*™ 3-color Prestained Protein Size marker (Broad)
Others	
K-2631	*AccuPower*® *ProFi Taq* PCR PreMix
K-3038	*AccuPrep*® PCR/Gel Purification Kit
K-3111	*AccuPrep*® Nano-Plus Plasmid Mini Extraction Kit
K-3122	*AccuPrep*® Nano-Plus Plasmid Midi Extraction Kit
K-3131	*AccuPrep*® Nano-Plus Plasmid Maxi Extraction Kit
K-3030	*AccuPrep*® Plasmid Mini Extraction Kit

Service information

Cat. No.	Service Description
S-2041	Gene Synthesis Service
S-6010	Gene Cloning Service

* For Custom Services, please inquire to order, or enter BIONEER's website to order.

Email address for inquiry: geneorder@bioneer.co.kr **BIONEER's website:** www.bioneer.com

Tel. 1588-9788 for Customer Service

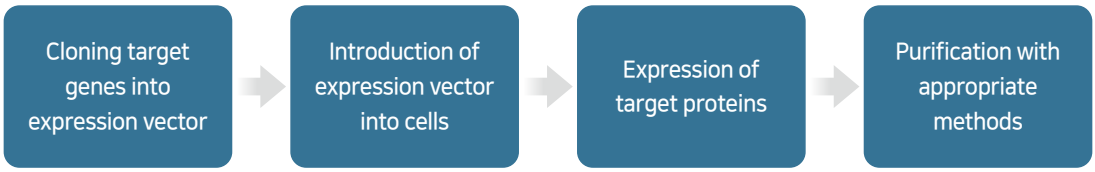
Part. IV

Protein Synthesizer and Purification

Introduction

Proteins include enzymes, hormones, structural and regulatory proteins, etc. and they play key roles in biological systems. With greater understanding of genome sequences, many researchers are trying to elucidate their functions and structures. The most critical step in the characterization of protein functions and features is the production of the protein. Below workflow shows the conventional way of producing a protein, using cells.

Conventional workflow of protein production:



The most common vessel for protein production has been cells such as *E. coli*, yeast or mammalian cells. However, there are serious drawbacks when using conventional method as listed below:

Disadvantages of conventional protein production method

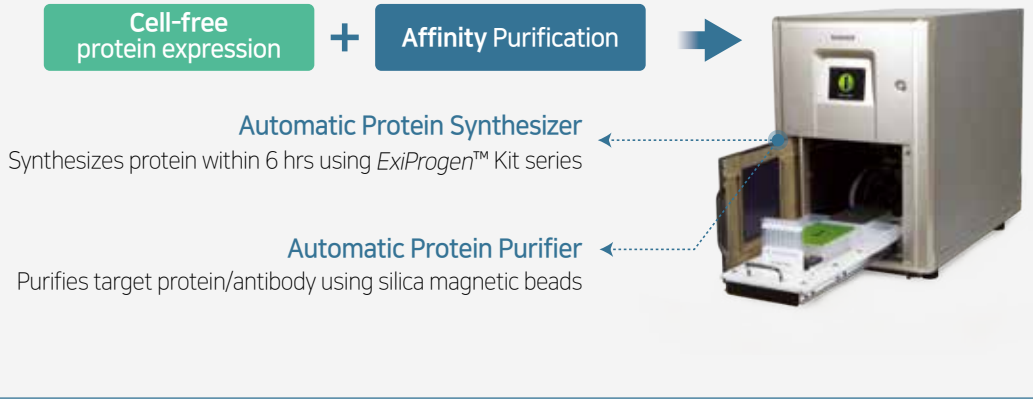
1) **Time-consuming and labor-intensive**
   - It takes a lot of time to select strains and search for suitable expression conditions
   - Must go through a series of processes to obtain protein, such as cell culture→cell disruption→ protein purification
   - Each process must be optimized

2) **Limited synthesis of some protein types**
   - Proteins that are toxic to cell lines inhibit cell growth and stable protein structure formation
   - When the intracellular environment is not suitable, the protein forms an inclusion body.

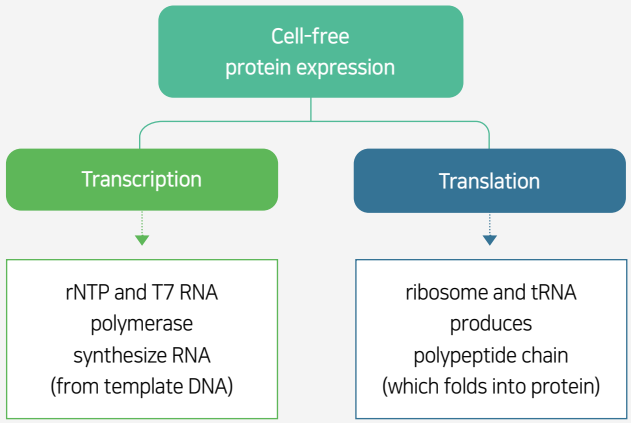
To overcome the limitations of cell-based system, cell-free protein expression method and related products have been developed. Cell-free protein expression system is a technology for protein expression by transcribing and translating *in vitro* based on cell extract. Because it is cell-free, it is not affected by cell growth and since the reaction proceeds in an open system, expression environment suitable for the target protein can easily be created.

BIONEER currently has various choice of protein synthesis and purification kits based on *E. coli* cell-free protein expression system. *ExiProgen*™'s cell-free protein expression system uses T7 RNA polymerase, enabling any gene of interest under the control of T7 promoter can be expressed using *ExiProgen*™.

Principles of Protein Synthesis and Purification



Cell-free Protein Expression

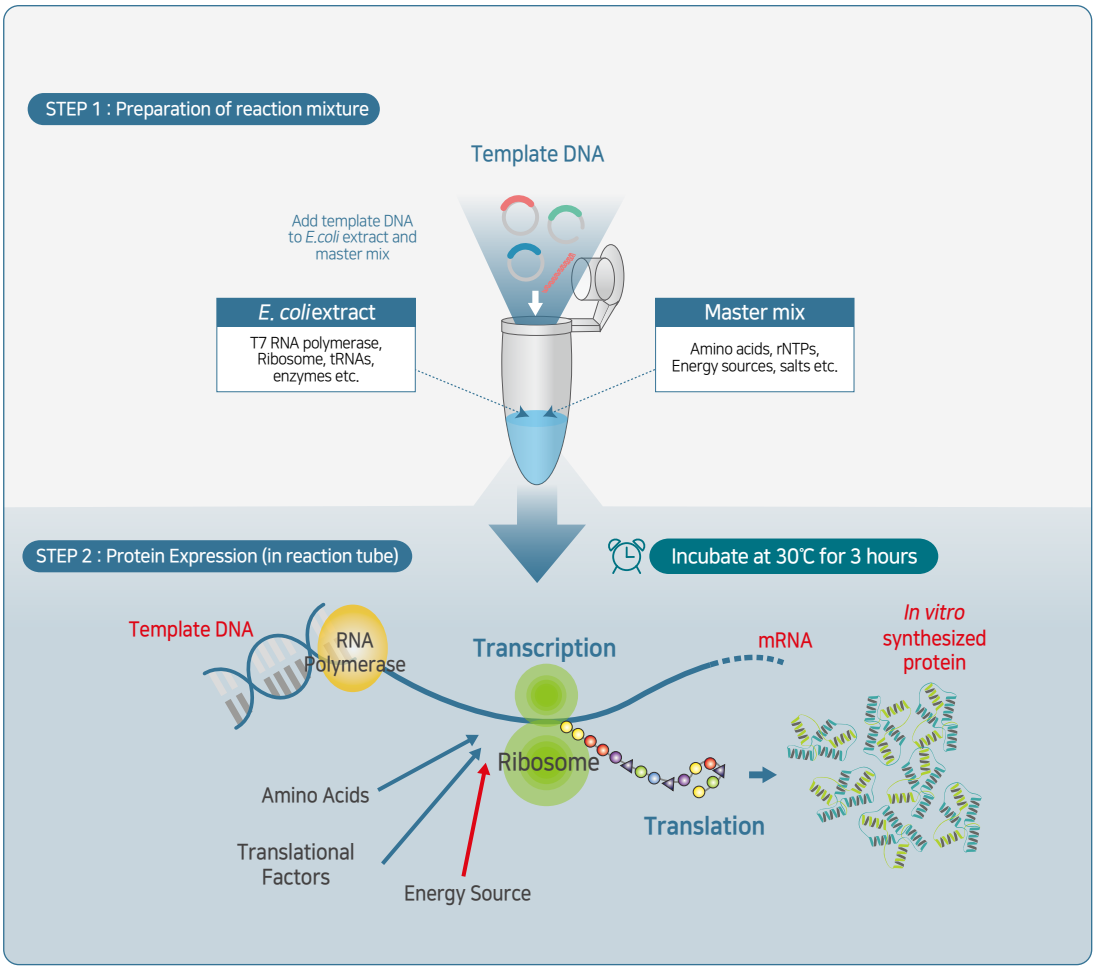


BIONEER's cell-free protein expression is a combined reaction of *in vitro* transcription and translation based on *E. coli* cell lysate. During transcription, rNTP, T7 RNA polymerase, etc. synthesize RNA from template DNA, and ribosome, tRNA, etc. are required for the translation step.

BIONEER's reaction mixture for cell-free protein expression includes *E. coli* cell extract (containing T7 RNA polymerase, ribosome and tRNA), and master mix (containing rNTP, amino acids and energy source) as illustrated in the next page.

In the presence of template DNA under the control of T7 promoter at 30℃, the reaction mixture triggers protein expression, where DNA sequence transcription starts and produces mRNA sequence. It is then followed by translation of the mRNA sequence into a protein using amino acids and energy source.

The diagram below illustrates the process of protein expression in two steps.



Affinity Purification with Magnetic Beads

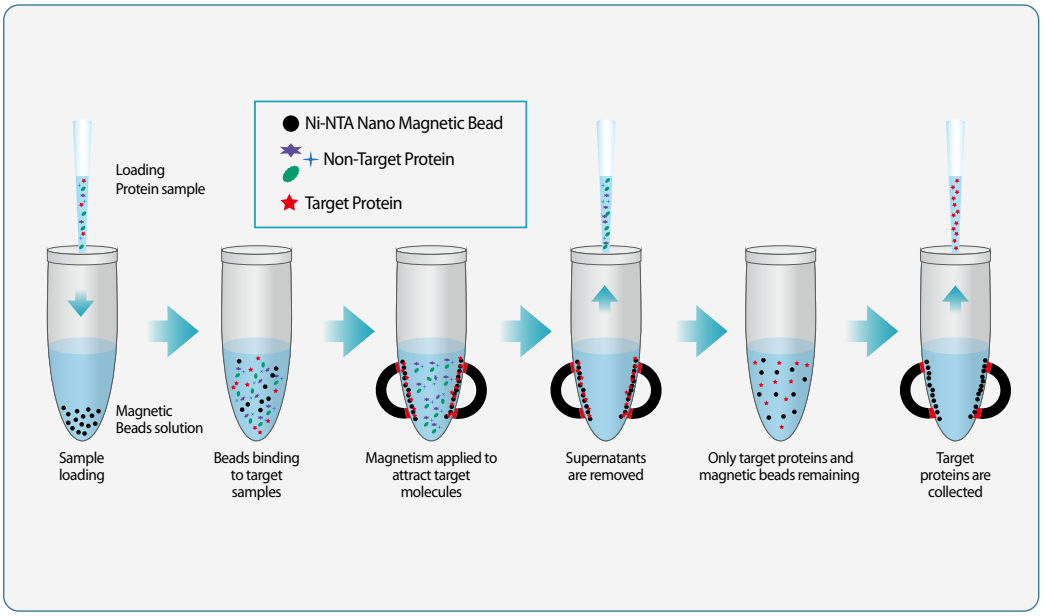
Purification of proteins such as antibodies, enzymes, hormones, structural proteins, and etc. contributes to many important biological and pharmaceutical applications. Among various protein purification methods, affinity chromatography is widely used because they ensure rapid access to recombinant proteins. In addition, the use of magnetic bead has outstanding advantages, allowing simple, fast, and high-throughput purification.

ExiProgen™ has applied these two methods for protein purification: **Affinity purification with magnetic beads**, having many advantages over existing resins and better suited for automation of the process. *ExiProgen*™ has applied these two methods for protein purification: Affinity purification with magnetic beads, having many advantages over existing resins and better suited for automation of the process. *ExiProgen*™ kit series for protein synthesis and purification use BIONEER's Ni-NTA magnetic beads, which are coated with functional group complementary to that of the target protein, allowing the beads to interact with biomolecules and bind together.

The *ExiProgen*™ can purify His-tagged proteins using Ni-NTA affinity. Every purification step is performed automatically by the instrument using Ni-NTA magnetic beads.

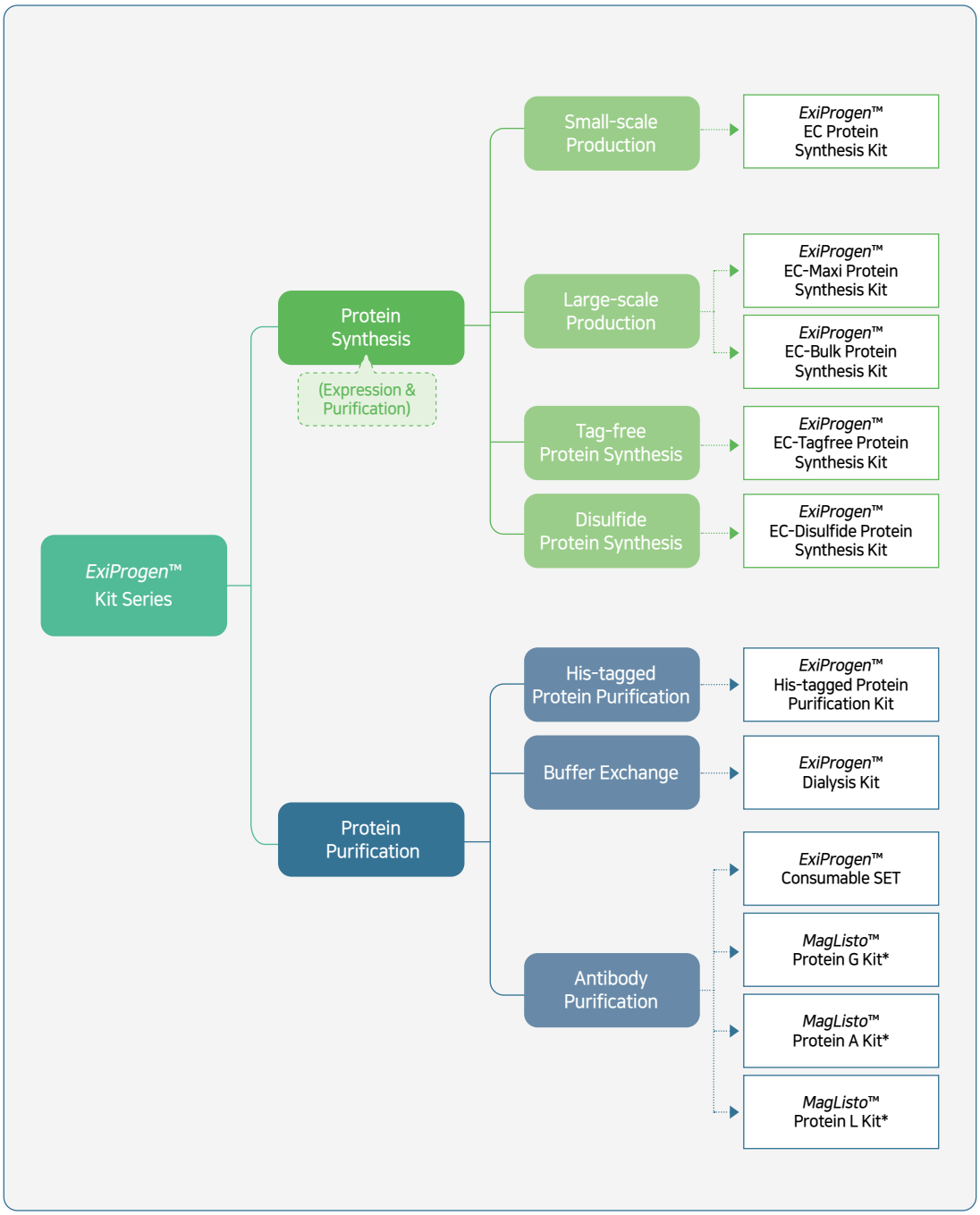
The procedure is as shown below:

1. Load protein sample to the magnetic beads solution.
2. Magnetic particles capture the His-tag within target protein.
3. Magnetism is used to attract the magnetic particles.
4. Washing buffer is used to wash away unbound molecules.
5. By adding imidazole as elution buffer, the target protein becomes unbound.
6. Purified target protein is obtained in an elution buffer.



Kit Selection Guide for Protein Synthesis and Purification

This selection guide helps to choose a suitable kit for protein synthesis or purification or dialysis, according to the required scale and type.



* *MagListo*™ Kits are manual kits, however when used with Consumable SET [Cat. No. KA-3001], they can be automated and run through *ExiProgen*™ instrument.

Checklist: Optimizing Protein Expression Level

I would like to check for the:

- ☐ Effect of an Optimized Codon → Go to page. 091
- ☐ Effect of DNA Purity on Protein → Go to page. 092
- ☐ Effect of Reaction Temperature on Protein → Go to page. 093
- ☐ Optimal Protein Expression Condition → Go to page. 094
- ☐ Effect of Batch Expression vs. Continuous Expression → Go to page. 097
- ☐ Possible Improvement on Protein Expression Level → Go to page. 098

By going through the checking points above, where appropriate, protein expression could be optimized and failure rates be lowered. Each checking point leads to Chapter 3 of this Guide Book, where a detailed explanation with experimental evidence is available.

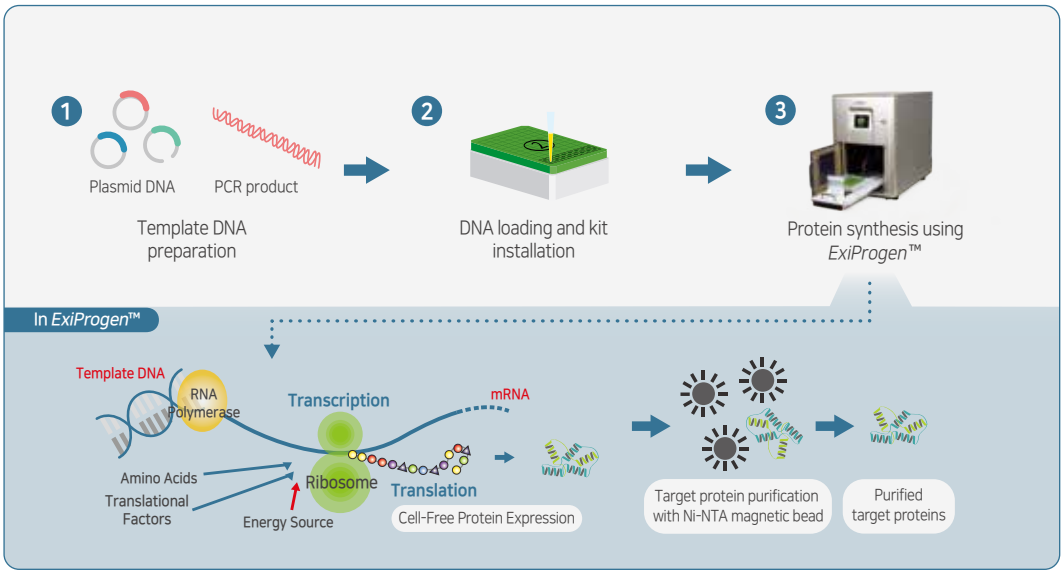
Chapter 1 - Automatic Protein Synthesis

***ExiProgen*™ Protein Synthesis Kit series** is used with BIONEER's fully-automated protein synthesizing instrument, which enables convenient protein synthesis and purification. It synthesizes proteins using cell-free protein expression system based on *E. coli* lysate, and purifies proteins using Histidine tag and Ni-NTA affinity, all in an automated process. Once template DNA is ready, various proteins can be synthesized in a rapid, easy, and convenient way.

- ✓ Synthesis of difficult-to-express proteins such as toxic proteins, membrane proteins, etc. made easy.
- ✓ Wide variety of samples (either plasmid DNA or PCR products) ranging from 10 to 150 kDa.
- ✓ All reagents are pre-dispensed into a buffer cartridge system.
- ✓ Ensures high yield and purity with BIONEER's patented magnetic nanobeads.
- ✓ Synthesize protein from up to 16 different samples simultaneously.
- ✓ Guarantees reproducible results with automated system.

▶ Procedure

1. Prepare template DNA (plasmid DNA or PCR product).
2. Load the template DNA and setup the instrument.
3. Select the correct Protocol No. and run the instrument.



* Note: BIONEER's cell-free protein synthesis product line consists of two classes:

1. *ExiProgen*™ Kit series for **automated** protein expression and purification
2. *AccuRapid*™ Kit series for **manual** expression and purification of proteins

This Part of Guide Book focuses on providing information of ***ExiProgen*™ Kit** series **ONLY**, as *AccuRapid*™ Kit series are not used with the instrument *ExiProgen*™.

1.1. Synthesizing up to 16 different proteins at a time

ExiProgen™ EC Protein Synthesis Kit

[Cat. No. K-7300 / K-7301 / K-7302]

This kit enables 16 different pure proteins to be obtained in a single experiment, simply by dispensing 16 different template DNA onto each well. This rules out tedious manual processing for individual protein. Purified target protein can be collected in an elution buffer in the elution tube.

Synthesis Amount	Up to 100 μg
Protocol No.	902
Running Time	6 hours

* Note: Each Cat. No. contains different number of kits; [K-7300/01/02: 1/2/6 kits]

▶ Features and Benefits

- **Fully automated system**
  Automatic expression & purification of the target protein only within 6 hours simply by inserting the template DNA into the cartridge and loading it to the *ExiProgen*™.
- **Advanced protein synthesis system**
  Synthesis of complicated proteins difficult to be done in traditional *in vivo* expression systems such as toxic proteins.
  Easy addition of additives such as chemicals and liposomes to create an appropriate expression environment for target proteins that are difficult to synthesize, such as membrane proteins.
- **Diversity**
  Protein expression of wide size ranges from 10 kDa to 150 kDa from a variety of DNA including plasmid DNA and PCR products.
- **Reproducibility**
  Reproducible results through optimized protocols of automated devices.

▶ Experimental Data

1.

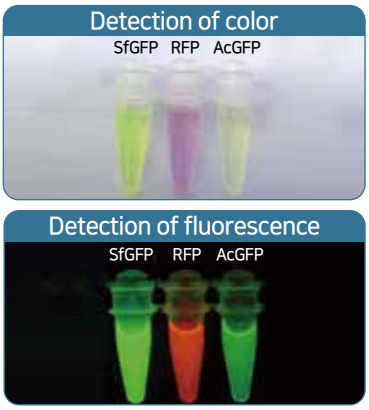


Figure 1. Detection of fluorescence emitted from fluorescent proteins synthesized with *ExiProgen*™.

The colors indicate that the synthesized SfGFP, RFP, and AcGFP are functionally active.

Top: Color of each protein elution samples observable with naked eyes.

Bottom: Fluorescence from protein elution samples detected with UV illuminator.

2.

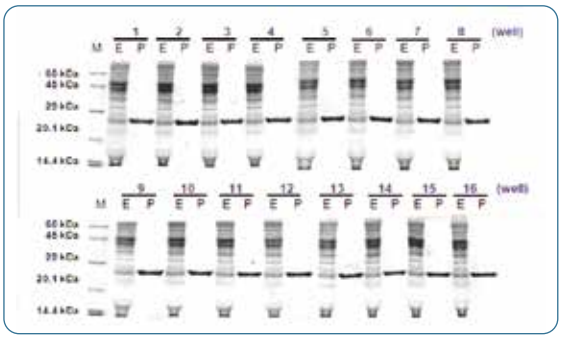


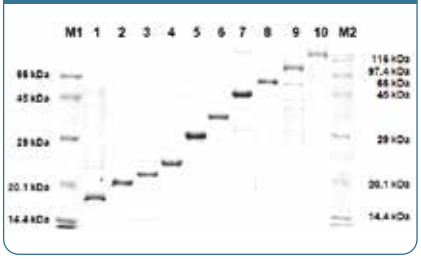
Figure 2. Expression and purification of the CAT enzyme. Reproducibility is seen in all 16 wells with no detectable variation between wells.

Lane 1-16: Number of the well
M: Protein Size Marker
E: Expression sample
P: Purification sample

3. It can successfully synthesize various proteins with different sizes.

Size range:
| Plasmid DNA: 10-120 kDa | PCR product: 10-60 kDa |

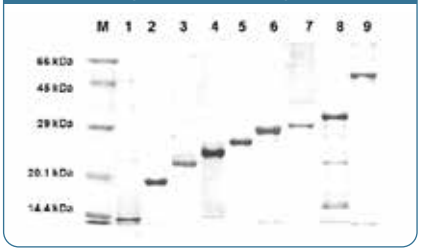
Plasmid DNA as a template



M1: Protein Size Marker 1 (Low)

Lane 1: CalmL3 (17.5 kDa)
Lane 2: RNase H (20 kDa)
Lane 3: DUSP 3 (22 kDa)
Lane 4: CAT (24 kDa)
Lane 5: AcGFP (29 kDa)
Lane 6: EF-Ts (34 kDa)
Lane 7: VF (45 kDa)
Lane 8: Poly A polymerase (50 kDa)
Lane 9: M-MLV RTase (75 kDa)
Lane 10: BM3 (117 kDa)
M2: Protein Size Marker 2 (Broad)

PCR product as a template



M: Protein Size Marker

Lane 1: SAV (13 kDa)
Lane 2: RNase H (20 kDa)
Lane 3: hGH (23 kDa)
Lane 4: CAT (26.5 kDa)
Lane 5: UDG (28 kDa)
Lane 6: AcGFP (28 kDa)
Lane 7: EVO (30 kDa)
Lane 8: RFP (31 kDa)
Lane 9: Poly A polymerase (54 kDa)

Figure 3. SDS-PAGE data of various proteins synthesized from different templates (Plasmid DNA & PCR products).
Up to 16 types of proteins can be expressed and purified simultaneously with an average of over 90% purity. Amount of loading sample is 1/35 of the total sample.

4. The final protein retains functional activity.

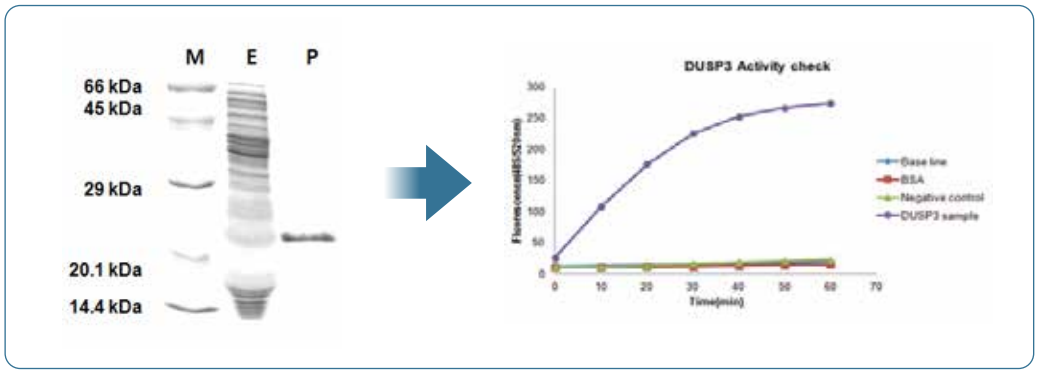


Figure 4. SDS-PAGE & enzyme activity data of DUSP3 (Dual Specificity Phosphatase 3).

Left: Expression and purification of DUSP3.

M: Protein Size Marker; E: Expression sample; P: Purification sample

Right: DUSP3 synthesized with *ExiProgen*™ showing enzyme activity.

DUSP3 phosphatase activity was measured by incubating with 500 μM 3-O-methylfluorescein phosphate (OMFP).

(Reaction buffer: 100 mM Tris-HCl, 40 mM NaCl, 1 mM DTT, 20% Glycerol, pH 8.2)

5.

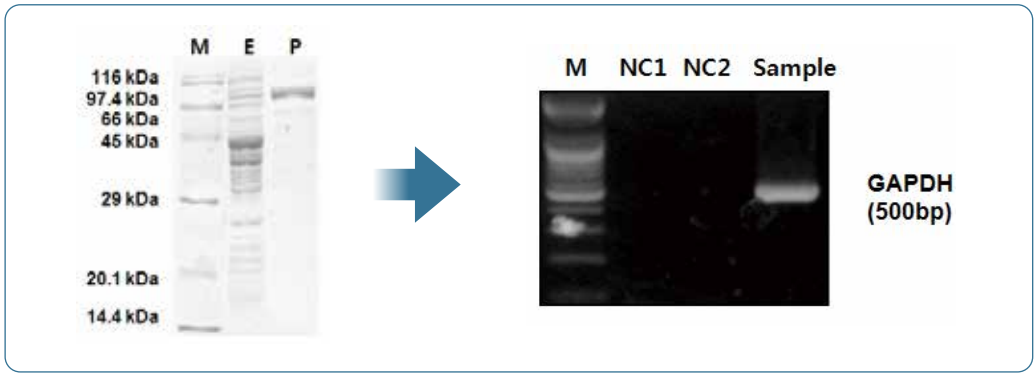


Figure 5. SDS-PAGE & enzyme activity data of M-MLV Reverse Transcriptase (RTase).

Left: Expression and purification of M-MLV RTase.

M: Protein Size Marker; E: Expression sample; P: Purification sample

Right: Amplification of GAPDH of Human total RNA with M-MLV RTase was synthesized with *ExiProgen*™.

M: 100 bp DNA Ladder; NC1: Negative control 1 (No-RNA);

NC2: Negative control 2 (No-enzyme); Sample: 10 ng of Human total RNA from HeLa cell

1.2. Obtaining up to 500 µg of protein

ExiProgen™ EC-Maxi Protein Synthesis Kit

[Cat. No. K-7310]

This kit is suitable for large-scale synthesis of target protein and uses the patented SECF (Stepwise Exchange Cell-Free) protein expression technology. Purified target protein can be collected as eluted in a storage buffer of choice.

Synthesis Amount	Up to 500 µg
Protocol No.	903
Running Time	25 hours

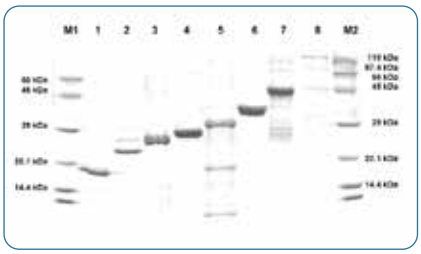
* Note: BIONEER highly recommends using **plasmid DNA** as a template DNA for this kit.

▶ Features and Benefits

- **Fully automated system**
  Automatic expression & purification of the target protein with yield of up to 500 µg only within 25 hours.
- **One-step multiprotein expression, purification, and dialysis**
  Expression, purification and dialysis of up to 8 different kinds of proteins simultaneously.
- **Advanced protein synthesis system**
  Synthesis of complicated proteins difficult to be done in traditional *in vivo* expression systems such as toxic proteins.
  Easy addition of additives such as chemicals and liposomes to create an appropriate expression environment for target proteins that are difficult to synthesize, such as membrane proteins.
- **Reproducibility**
  Reproducible results through optimized protocols of automated devices.

▶ Experimental Data

Up to eight proteins with various sizes can be synthesized in a single run.



M1: Protein Size Marker 1 (Low)
Lane 1: CalmL3 (17.5 kDa)
Lane 2: DUSP 3 (22 kDa)
Lane 3: CAT (24 kDa)
Lane 4: AcGFP (29 kDa)
Lane 5: RFP (30 kDa)
Lane 6: EF-Ts (34 kDa)
Lane 7: VF (45 kDa)
Lane 8: BM3 (117 kDa)
M2: Protein Size Marker 2 (Broad)

Figure 1. Expression and purification of various proteins.
Proteins can be expressed and purified simultaneously with an average of over 90% purity. Amount of loading sample is 1/80 of total sample.

HIT!

1.3. Obtaining up to 10 mg of protein

ExiProgen™ EC-Bulk Protein Synthesis Kit

[Cat. No. K-7340]

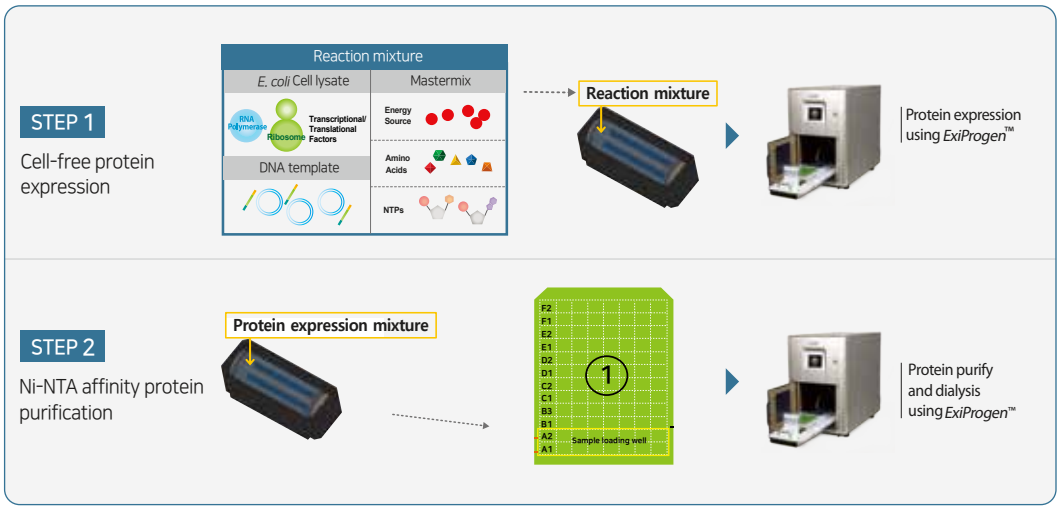
This kit is used to synthesize protein in a bulk scale with the patented SECF (Stepwise Exchange Cell-Free) technology, which maximizes protein expression level through continuous reagents supply.

Synthesis Amount	Up to 10 mg
Protocol No.	906 & 907
Running Time	40 hours

▶ Features and Benefits

- **Productive**
  Obtain up to 10 mg of purified protein per reaction.
- **Quick & Convenient**
  Synthesize your proteins automatically without the process of cell culturing and protein purification, which is time and labor-consuming.
- **Advanced protein synthesis system**
  Synthesis of complicated proteins difficult to be done in traditional *in vivo* expression systems such as toxic proteins.
  Easy addition of additives such as chemicals and liposomes to create an appropriate expression environment for target proteins that are difficult to synthesize, such as membrane proteins.
- **Reproducibility**
  Reproducible results through optimized protocols of automated devices.

▶ Procedure



STEP 1 **The protein expression process takes about 25 hours**

1. Add template DNA, *E. coli* extract and master mix to make a reaction mixture.
2. Fill the bulk reactor with the reaction mixture as prepared above.
3. Install the bulk reactor and other components and select Protocol No. 906.

STEP 2 **The protein purification process takes about 15 hours**

1. Load the expressed protein to sample loading well.
2. Replace the bulk reactor with a new one and fill with sterile distilled water.
3. Select Protocol No. 907 and run the instrument.

▶ Experimental Data

1. Synthesis of fluorescent protein

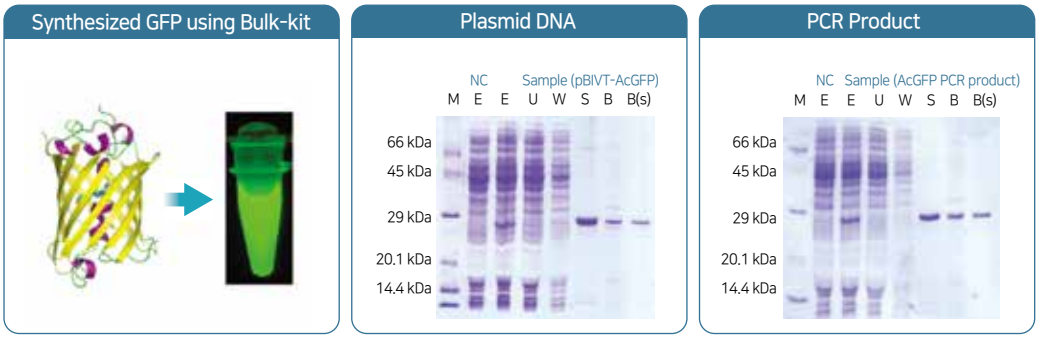


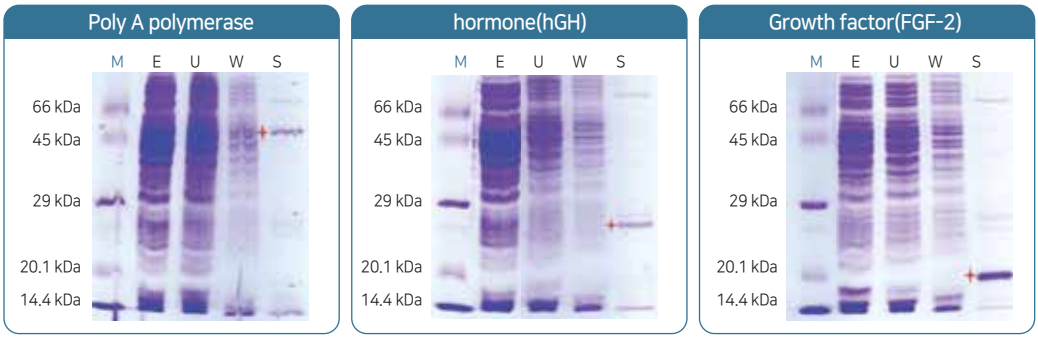
Figure 1. Positive control protein synthesis data using *ExiProgen*™ EC-Bulk Protein Synthesis Kit.

Plasmid DNA: pBIVT-AcGFP used, PCR Product: AcGFP PCR product used.

NC: Negative control M: Protein Size Marker

E: Expression sample U: Unbound sample W: Washing sample

S: Storage sample B: Bead sample B(s): Supernatant of bead sample



2. Synthesis of various proteins

Figure 2. High-value protein synthesis data using *ExiProgen*™ EC-Bulk Protein Synthesis Kit.

M: Protein Size Marker E: Expression sample U: Unbound sample

W: Washing sample S: Storage sample

1.4. Obtaining His-tag-removed protein

ExiProgen™ EC-Tagfree Protein Synthesis Kit

[Cat. No. K-7320]

This kit produces the final protein without His-tag. A protein with His-tag is first expressed. Then, the His-tag is cleaved off, resulting in a pure protein without a His-tag sequence. Purified target protein can be collected as eluted in a storage buffer of choice.

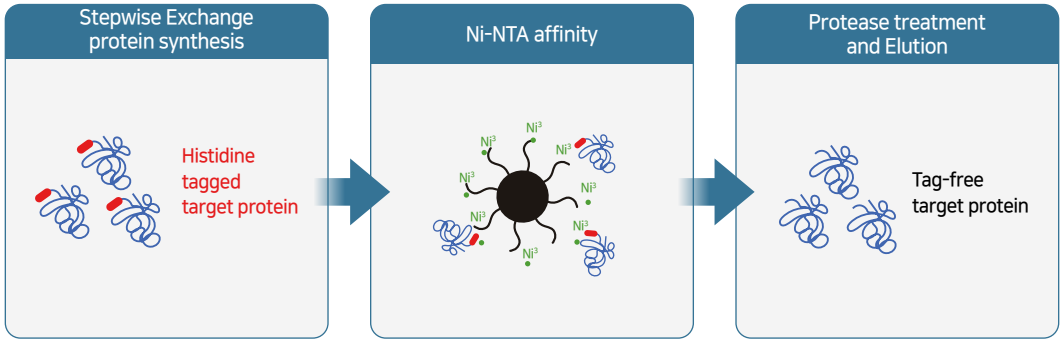
Synthesis Amount	Up to 200 µg
Protocol No.	904
Running Time	26 hours

▶ Features and Benefits

- **Histidine-tag removed protein recovery**
  Protein purification followed by removal of histidine-tags.
- **Fully automated system**
  Automatic expression & purification of the target protein only within 26 hours.
- **Concurrent multiprotein expression/purification/dialysis**
  Simultaneous expression, purification, and dialysis of 1-8 different kinds of proteins.
- **Advanced protein synthesis system**
  Synthesis of complicated proteins difficult to be done in traditional *in vivo* expression systems such as toxic proteins.
  Easy addition of additives such as chemicals and liposomes to create an appropriate expression environment for target proteins that are difficult to synthesize, such as membrane proteins.
- **Reproducibility**
  Reproducible results through optimized protocols of automated devices.

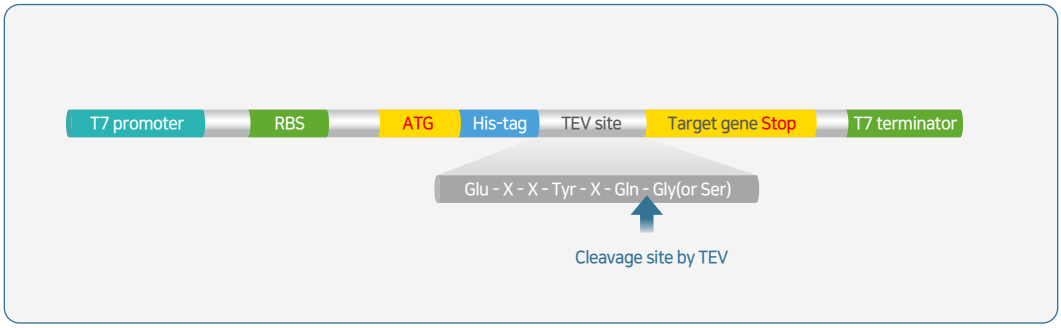
▶ Procedure

This kit is for an automated expression of a target protein using patented SECF (Stepwise Exchange Cell-Free) technology. The expressed protein is bound to Ni-NTA magnetic beads and then released by cleaving with TEV (Tobacco Etch Virus) protease that recognizes specific amino acid sequence.



▶ The structure of Template DNA

The template DNA elements should be organized as follows.



The His-tag should be at 5' end of the target protein and TEV cleavage sequence should be placed in between the His-tag and the target gene.

The resulting purified protein will have only one extra amino acid (Glycine or Serine) at the N-terminus of the target protein. The position X can be any of 20 amino acids, while the DNA sequence provided as a positive control encodes Glu-Asn-Leu-Tyr-Phe-Gln-Gly.

▶ Experimental Data

1. Various proteins can be synthesized in a single run. The yield will be up to 200 µg per well.

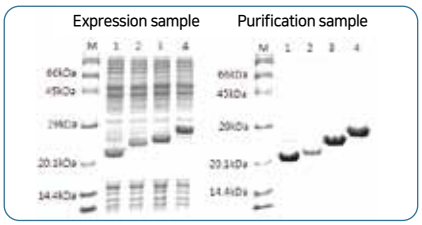


Figure 1. Expression and purification of various proteins

M: Protein Size Marker
Lane 1: DUSP3 (22 kDa)
Lane 2: hGH (23 kDa)
Lane 3: AcGFP (28 kDa)

* Note: DUSP3, Dual specificity protein phosphatase 3; hGH, Human growth hormone.

2. The target protein will have the His-tag removed.

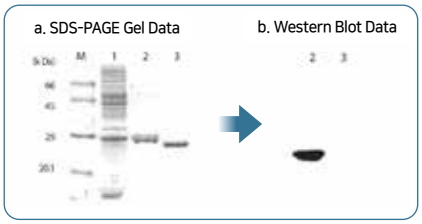


Figure 2. Data of SDS-PAGE and Western blot with His-tag antibody

M: Protein Size Marker
Lane 1: Expression sample of pBIVT-TEV-AcGFP
Lane 2: His tag-TEV-AcGFP (**His-tagged sample**)
Lane 3: AcGFP (**His tag-free sample**)

1.5. Obtaining Disulfide bond-containing protein

ExiProgen™ EC-Disulfide Protein Synthesis Kit

[Cat. No. K-7330]

This kit is used for synthesizing a protein that contains disulfide bonds. Used in conjunction with *ExiProgen*™, it can synthesize a protein that contains more than two and up to nine disulfide bonds in a fully automated manner with higher efficiency than cell-based expression. Purified target protein can be collected as eluted in a storage buffer of choice.

Synthesis Amount	Up to 100 µg
Protocol No.	905
Running Time	36 hours

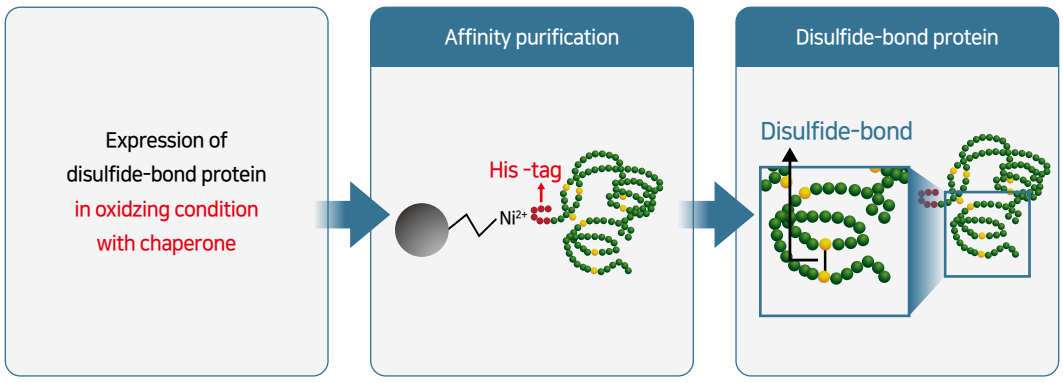
▶ Features and Benefits

- **Disulfide bond protein synthesis**
  Regulation of the protein expression environment to form disulfide bonds.
- **Fully automated system**
  Automatic expression & purification of the target protein only within 36 hours.
- **Concurrent multiprotein expression/purification/dialysis**
  Simultaneous expression, purification, and dialysis of 1-8 different kinds of proteins with disulfide bonds.
- **Reproducibility**
  Reproducible results through optimized protocols of automated devices.

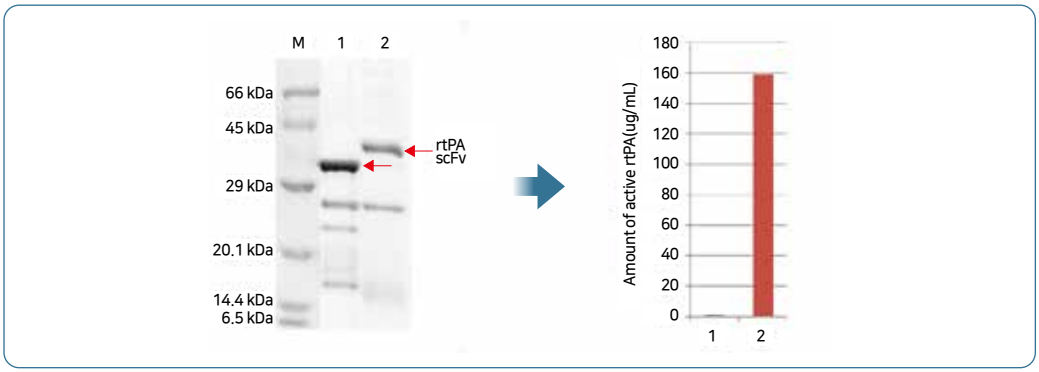
▶ Principle

This product contains a buffer solution that increases the redox potential for optimal disulfide bond formation and chaperone that helps to form a protein structure with disulfide bonds. In addition, our SECF technology is applied to simultaneously remove impurities during protein expression and to continuously supply substrates and energy sources to create an optimal protein expression environment.

The expressed protein is then purified by the affinity method using His-tag, and the final target protein can be recovered in a dissolved state in the storage buffer.



▶ Experimental Data



1. This kit is capable of synthesizing proteins with disulfide bonds and their activities are tested.

Figure 1. SDS-PAGE & enzyme activity data.

Left: Expression and purification of ScFV and rPA. Amount of purification sample is 1/40 of total sample.

M: Protein Size Marker

Lane 1: ScFV (it has 2 disulfide bonds)

Lane 2: rPA (it has 9 disulfide bonds)

Right: rPA synthesized has an enzyme activity. rPA activity was measured by incubating with chromogenic substrate S-2288.

Sample 1: Synthesis sample using *ExiProgen*™ EC-Maxi Protein Synthesis Kit.

Sample 2: Synthesis sample using *ExiProgen*™ EC Protein Synthesis Kit.

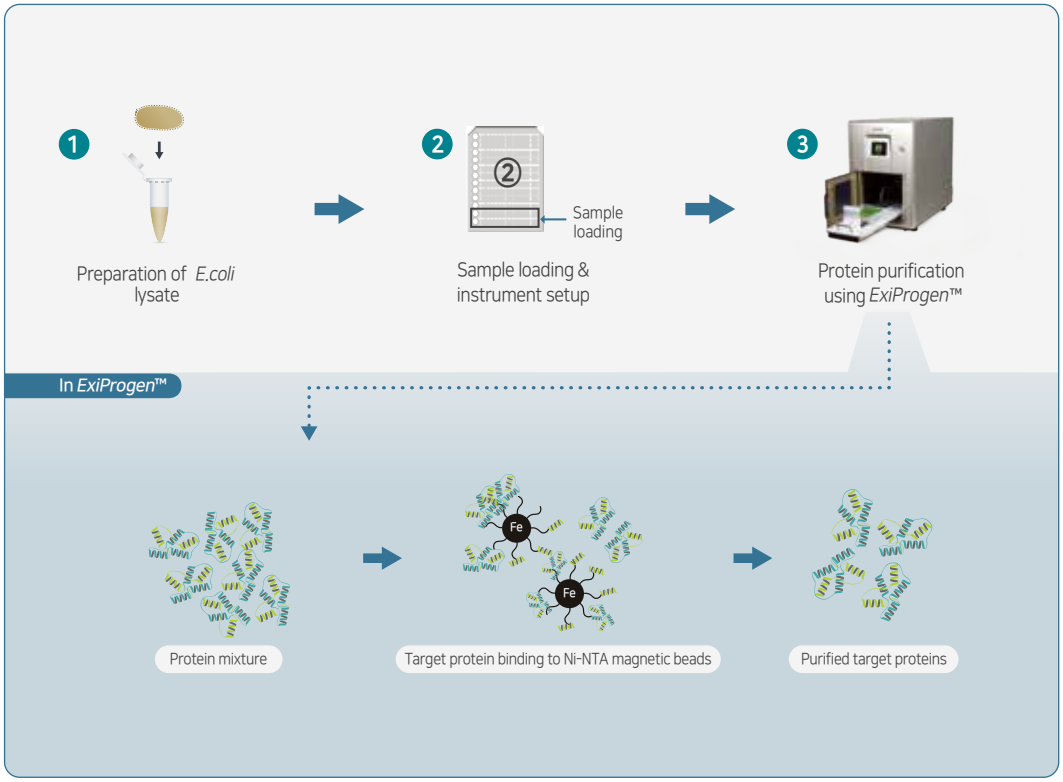
Chapter 2 - Automatic Protein Purification

ExiProgen™ Protein Purification Kit contains patented silica magnetic bead, which is coated with either Ni-NTA or with Protein G/A/L that can purify targets containing His-tags or antibodies, respectively.

- ☑ A wide range of purification is possible for all recombinant proteins with His-tag or antibodies with protein G/A/L expressed *in vivo* or *in vitro* system.
- ☑ Purify up to 16 different proteins at one time with over 90% purity.
- ☑ Can easily exchange buffer to a desired buffer using *ExiProgen*™ Dialysis Kit.
- ☑ Pre-filled cartridge contains all reagents required for protein purification which simplifies the process and reduces the variation between reactions.

In just three steps, the target protein can be purified in a fully-automated manner.

▶ Procedure



1. Prepare and pre-treat the sample.
2. Load the sample and setup the instrument.
3. Select the correct Protocol No. and run the instrument.

2.1. Purification of His-tagged protein

ExiProgen™ His-tagged Protein Purification Kit [Cat. No. K-7220 / K-7221]

This kit is used to purify target protein with His-tag, from *E. coli* lysate or cell-free protein expression reaction mixture. The kit contains Ni-NTA magnetic beads and all other reagents as a pre-filled cartridge. 500 µl of Ni-NTA magnetic beads can bind with 1.5-2 mg of His-tagged protein, and up to 16 different types of protein can be simultaneously purified.

Protocol No.	901
Running Time	2 hours

▶ Features and Benefits

- **Broad compatibility**
  A wide range of purification ability: All recombinant proteins with His-tag expressed *in vivo* protein expression in *E. coli* or *in vitro* (cell-free protein expression).
- **High binding capacity**
  Around 3-4 mg of 6x His-tagged protein can bind onto 1 ml of Ni-NTA magnetic nanobead. Ni-NTA bead is pre-dispensed onto well plate for about 500 µl (with 10% bead volume), so each well has binding capacity of around 1.5-2 mg.
- **Fully automated system**
  User-friendly system, allowing easy manipulation of instrument using pre-installed protocol.
- **Concurrent multi-protein purification**
  Simultaneous purification of protein up to 16 different types.

▶ Experimental Data

1. Preparation of recombinant *E. coli* cell with expressed DUSP3 using *ExiProgen*™.

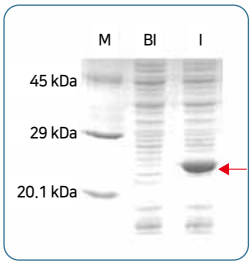


Figure 1. Recombinant *E. coli* cell with expressed DUSP3.
M: Protein Size Marker
BI: Pre-induction sample
I: Post-Induction sample

2. Target protein (DUSP3) purification using *ExiProgen*™.

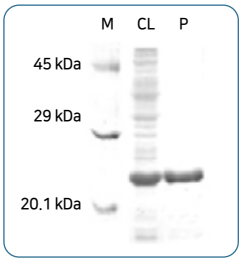
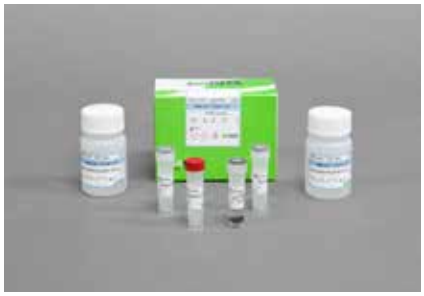


Figure 2. Purification of DUSP3 with *ExiProgen*™
M: Protein Size Marker
CL: Recombinant cell lysate sample
P: Purified sample

2.2. Purification of Antibody

MagListo™ Protein G Kit

[Cat. No. K-7710]



This kit allows rapid and easy purification of antibodies using magnetic separation method. It consists of Protein G Magnetic Nanobead and buffer for antibody purification. Magnetic Nanobeads are magnetic silica beads coated with high purity (> 95%) protein G. The coated protein G has affinity specific to a particular antibody structure, allowing purification of **1.2 mg of Human IgG per 1 ml of bead solution.**

Protocol No.	341
Running Time	< 1 hour

* Note: *MagListo*™ Kit series are manual type, and not automatic type. However, this kit can be automated when used in combination with the *ExiProgen*™ Consumable SET [Cat. No. KA-3001].

▶ Features and Benefits

- **Reduced experimental time**
  Rapid process with minimized loss using powerful magnetism of Magnetic Nanobeads.
- **High binding capacity**
  Large surface area of Magnetic Nanobeads with average diameter of 400 nm.
- **Strong specificity**
  Reduced non-specific binding through the use of homogeneous spherical nanobeads.

▶ Experimental Data

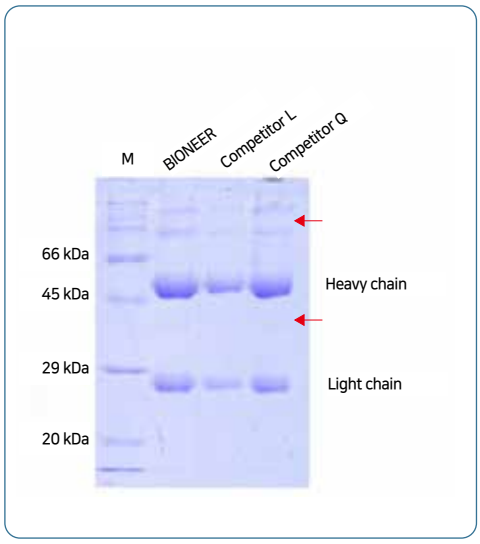
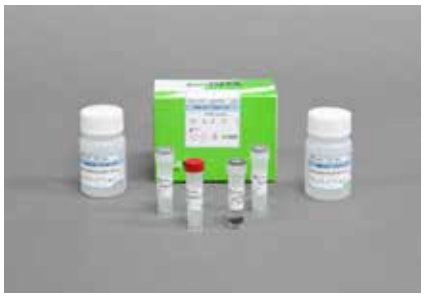


Figure 1. Comparison of protein binding efficiency of *AccuNanoBead*™ Protein G Magnetic NanoBeads (in *MagListo*™ Protein G Kit) with a competitor.
Elution fraction contains Human IgG purified with Protein G magnetic bead from BIONEER (*MagListo*™ Protein G Kit), a competitor L, and a competitor Q. Upper and lower band are heavy (HC) and light chain (LC) of purified Human IgG in SDS-PAGE gel, respectively.
M: Protein Size Marker.

MagListo™ Protein A Kit

[Cat. No. K-7720]



This kit allows rapid and easy purification of antibodies using magnetic separation method. It consists of Protein A Magnetic Nanobead and buffer for antibody purification. Magnetic Nanobeads are magnetic silica beads coated with high purity (> 95%) protein A. The coated protein A has affinity specific to a particular antibody structure, allowing purification of **800 µg of Human IgG per 1 ml of bead solution.**

Protocol No.	342
Running Time	< 1 hour

* Note: *MagListo*™ Kit series are manual type, and not automatic type. However, this kit can be automated when used in combination with the *ExiProgen*™ Consumable SET [Cat. No. KA-3001].

▶ Features and Benefits

- **Reduced experimental time**
  Rapid process with minimized loss using powerful magnetism of Magnetic Nanobeads.
- **High binding capacity**
  Large surface area of Magnetic Nanobeads with average diameter of 400 nm.
- **Strong specificity**
  Reduced non-specific binding through the use of homogeneous spherical nanobeads.

▶ Experimental Data

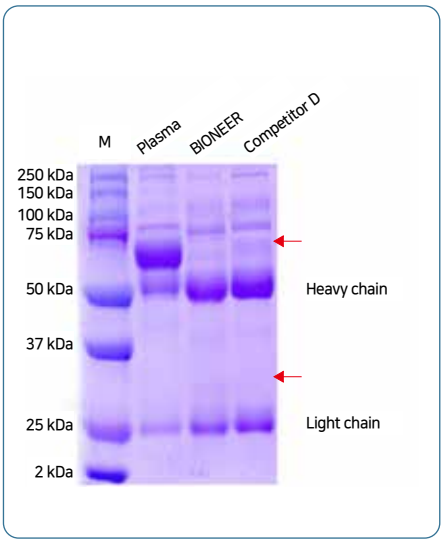
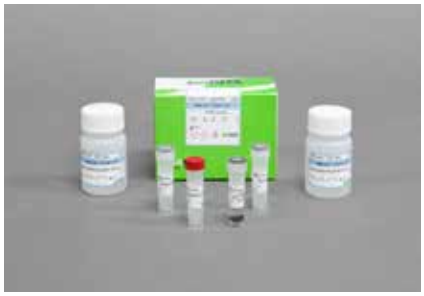


Figure 1. IgG purification from plasma with *AccuNanoBead*™ Protein A Magnetic NanoBead and Competitor D.
Eluted fraction after binding of Human IgG followed by washing through Protein A magnetic bead from BIONEER (*AccuNanoBead*™ Protein A Magnetic NanoBeads) and from competitor D. Upper and lower band are heavy (HC) and light chain (LC) of purified Human IgG in SDS-PAGE gel, respectively.
M: Protein Size Marker.

2.2. Purification of Antibody

MagListo™ Protein L Kit

[Cat. No. K-7730]



This kit allows rapid and easy purification of antibodies using magnetic separation method. It consists of Protein L Magnetic Nanobead and buffer for antibody purification. Magnetic Nanobeads are magnetic silica beads coated with high purity (> 95%) protein L. The coated protein L has affinity specific to a particular antibody structure, allowing purification of **800 µg of Human IgG per 1 ml of bead solution.**

Protocol No.	343
Running Time	< 1 hour

* Note: *MagListo*™ Kit series are manual type, and not automatic type. However, this kit can be automated when used in combination with the *ExiProgen*™ Consumable SET [Cat. No. KA-3001].

▶ Features and Benefits

- **Reduced experimental time**
  Rapid process with minimized loss using powerful magnetism of Magnetic Nanobeads.
- **High binding capacity**
  Large surface area of Magnetic Nanobeads with average diameter of 400 nm.
- **Strong specificity**
  Reduced non-specific binding through the use of homogeneous spherical nanobeads.

▶ Experimental Data

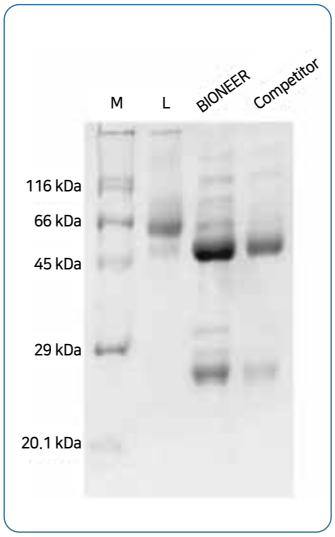


Figure 1. Purification of Human IgG with *MagListo*™ Protein L Kit.
Elution fraction contains human IgG purified with protein L magnetic bead from BIONEER. Upper and lower band are heavy (HC) and light chain (LC) of purified human IgG in SDS-PAGE gel, respectively.
M: Protein Size Marker
L: Human plasma

ExiProgen™ Consumable SET

[Cat. No. KA-3001]

This set is a disposable product, which can automate a manual purification process. It can be used with *MagListo*™ Protein G/A/L Kits described above, which are manual kits and *MagListo*™ PCR/Gel Purification Kit. when used with the instrument *ExiProgen*™, 1 to 16 samples can be simultaneously purified.

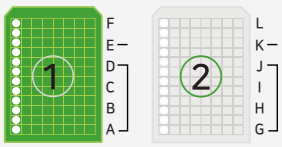
▶ Features and Benefits

- **Automated system**
  Pre-installed protocol allowing easy purification of antibodies.
- **Simultaneous multiple purification**
  Simultaneous purification of protein up to 16 different types.
- **Customizability**
  Desired amount of bead ranging from 1 to 20 mg can be used as needed.
- **Reproducibility**
  Reproducible experimental results obtained by using automatic dispensing system, minimizing error range.

▶ Procedure and Principle

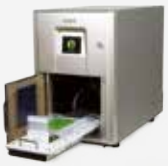
❶ Preparation of sample

❷ Sample loading & Instrument set-up



❸ Protocol Set-up

❹ Protein purification with *ExProgen*™



1. Load the sample, buffer, and the magnetic beads onto Cartridge ① and ②.
2. Install them on the instrument *ExiProgen*™, select the correct Protocol No. and run.

▶ Experimental Data

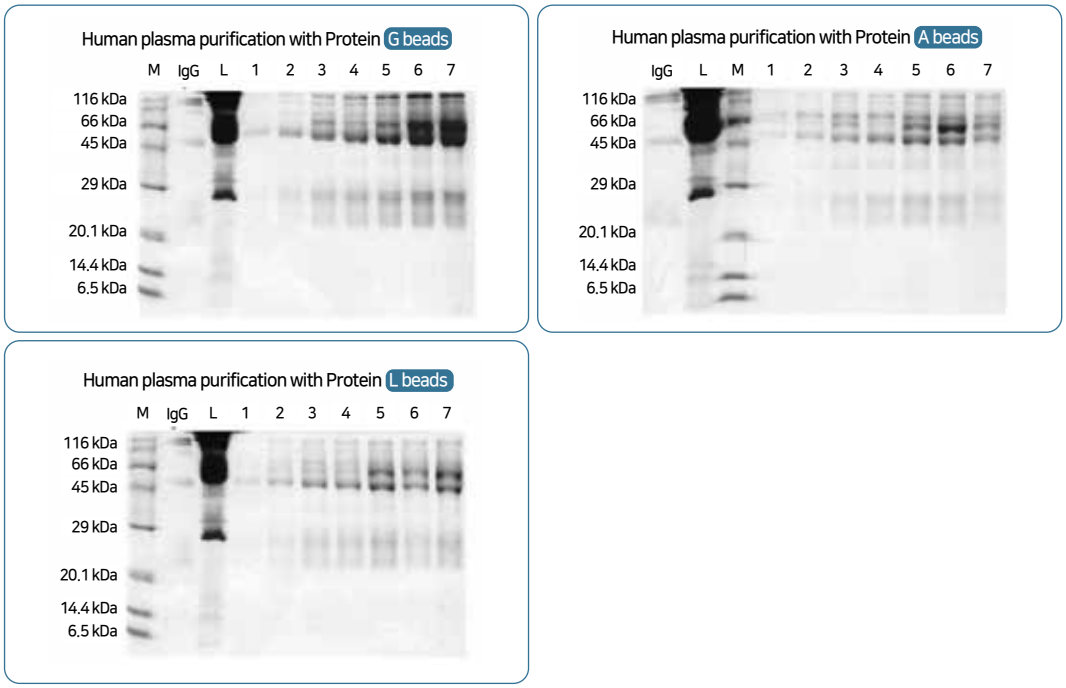


Figure 1. Human plasma purification using *MagListo*™ Protein G, Protein A, Protein L Kit and *ExiProgen*™ Consumable SET.
The volume of protein samples loaded into each well is 7.5 µl/400 µl total elution samples.
M:Protein Size Marker
IgG: Purified human IgG (3 µg)
L: Loading sample (human plasma, 500 µl)
Lane 1-7: Amount of beads (1, 2.5, 5, 7.5, 10, 15, 20 mg)

2.3. Dialysis of Purified Protein

ExiProgen™ Dialysis Kit

[Cat. No. K-7240]

This kit is used to exchange buffer using dialysis in a fully-automated way through the instrument *ExiProgen*™. Two protocols are available, each with different number of dialysis process. The number of exchange times and the sample volume can be chosen. Up to 16 different kinds of buffer can be exchanged simultaneously. The final targets then can be obtained dissolved in the desired buffer of your choice.

Protocol No.	Protocol name	Number of dialysis process	Sample volume
913	Storage_12	24 times	700 µl
914	Storage_6	12 times	700 µl

▶ Features and Benefits

- **High efficiency**
  Exchange with a desired buffer using a small amount of buffer.
- **Economic and user-friendly**
  Buffer exchange possible even at a low temperature without an additional cooling system.
- **Fully automated system**
  Once samples are loaded, all the remaining processes are automated.
- **Parallel Processing**
  Simultaneous exchange of buffers for 1~16 different proteins, each to an optimized buffer.

▶ Experimental Data

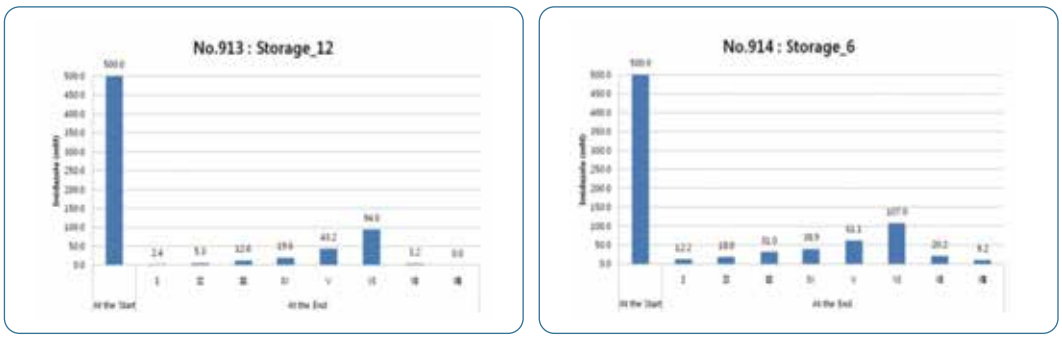


Figure 1. Comparison of dialysis efficiency according to different protocol using 500 mM imidazole.

Ⅰ : 50 mM Tris-Cl, 100 mM NaCl, 0.2 mM EDTA, 0.05% NaN_3, pH 7.6
Ⅱ : 50 mM Tris-Cl, 100 mM NaCl, 0.2 mM EDTA, 0.05% NaN_3, 10% Glycerol, pH 7.6
Ⅲ : 50 mM Tris-Cl, 100 mM NaCl, 0.2 mM EDTA, 0.05% NaN_3, 20% Glycerol, pH 7.6
Ⅳ : 50 mM Tris-Cl, 100 mM NaCl, 0.2 mM EDTA, 0.05% NaN_3, 30% Glycerol, pH 7.6
Ⅴ : 50 mM Tris-Cl, 100 mM NaCl, 0.2 mM EDTA, 0.05% NaN_3, 40% Glycerol, pH 7.6
Ⅵ : 50 mM Tris-Cl, 100 mM NaCl, 0.2 mM EDTA, 0.05% NaN_3, 50% Glycerol, pH 7.6
Ⅶ : PBS
Ⅷ : 100 mM HEPES, 100 mM NaCl, 0.2 mM EDTA, 0.05% NaN_3, pH 7.6

Summary of Kits for Protein Synthesis and Purification:

Kit type & Purpose

Product for protein synthesis	Cat. No.	Purpose
ExiProgen™ EC Protein Synthesis Kit	K-7300	Synthesize protein (up to 100 µg)
ExiProgen™ EC-Maxi Protein Synthesis Kit	K-7310	Synthesize protein (up to 500 µg)
ExiProgen™ EC-Bulk Protein Synthesis Kit	K-7340	Synthesize protein (up to 10 mg)
ExiProgen™ EC-Disulfide Protein Synthesis Kit	K-7330	Synthesize protein with disulfide bonds
ExiProgen™ EC-Tagfree Protein Synthesis Kit	K-7320	Synthesize protein without His-tags
Product for protein purification	**Cat. No.**	**Purpose**
ExiProgen™ His-tagged Protein Purification Kit	K-7220	His-tagged protein purification
MagListo™ Protein G Kit	K-7710	Antibody purification
MagListo™ Protein A Kit	K-7720	Antibody purification
MagListo™ Protein L Kit	K-7730	Antibody purification
Product for protein dialysis	**Cat. No.**	**Purpose**
ExiProgen™ Dialysis Kit	K-7240	Buffer Exchange

Protocol Number & Protocol Name

Product Description	Protocol No.	Protocol Name
ExiProgen™ His-tagged Protein Purification Kit	901	Protein Purification
ExiProgen™ EC Protein Synthesis Kit	902	Protein Synthesis
ExiProgen™ EC-Maxi Protein Synthesis Kit	903	Protein: Synthesis_Maxi
ExiProgen™ EC-Tagfree Protein Synthesis Kit	904	Protein: Synthesis_TF
ExiProgen™ EC-Disulfide Protein Synthesis Kit	905	Protein: Synthesis_DS
ExiProgen™ EC-Bulk Protein Synthesis Kit	906	Protein: Bulk_Expression
	907	Protein: Bulk_Purification
MagListo™ Protein G Kit	341	Purification: Protein_G
MagListo™ Protein A Kit	342	Purification: Protein_A
MagListo™ Protein L Kit	343	Purification: Protein_L
ExiProgen™ Dialysis Kit	913	Storage_12
	914	Storage_6

Chapter 3 - Optimizing protein expression level

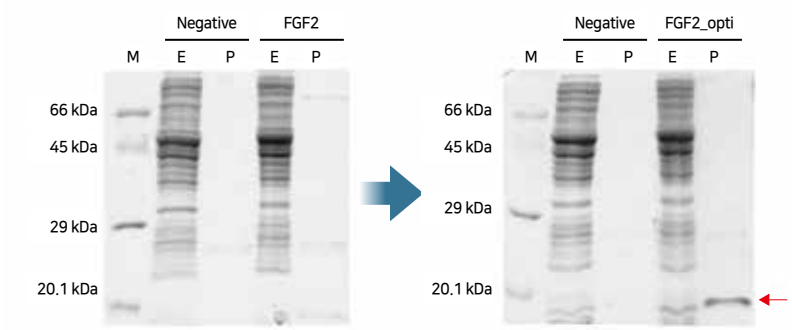
3.1. Codon Optimization for the maximal Protein Expression

When cell-free protein synthesis is used for expression of eukaryotic genes, protein yield might be low due to differences in codon usage from prokaryotic systems. In such cases, protein yield could be increased by codon optimization that steers the eukaryotic codon usage to *E. coli* preferred codons.

▶ Experimental Data

Yield of FGF-2 (Fibroblast Growth Factor-2), a eukaryotic gene, was greatly increased by optimizing codon usage. The following condition was used for the experiment.

Template DNA	pBIVT-FGF2, pBIVT-FGF2_opti.
Kit	*ExiProgen*™ EC Protein Synthesis Kit [Cat. No. K-7300]
Protocol No.	902
Sampling	Total 20 µl (Purification sample 15 µl + 4X loading dye 5 µl) → Boil at 95°C, 5 min
SDS-PAGE gel electrophoresis	Loaded 10 µl of boiled sample onto each well [10 × 8 (cm), 10 wells]



M: Protein Size Marker
Negative: Negative control (No-DNA)
FGF2: pBIVT-FGF2 (Original sequences)
FGF2_opti.: pBIVT-FGF2_opti. (*E. coli* codon optimized sequences)
E: Expression sample
P: Purification sample

3.2. Effect of Template DNA Purity on protein expression

Purity of template DNA affects the protein yield. BIONEER recommends the use of high purity DNA with $A_{260/280}$ > 1.7-2.0 and $A_{260/230}$ >1.5.

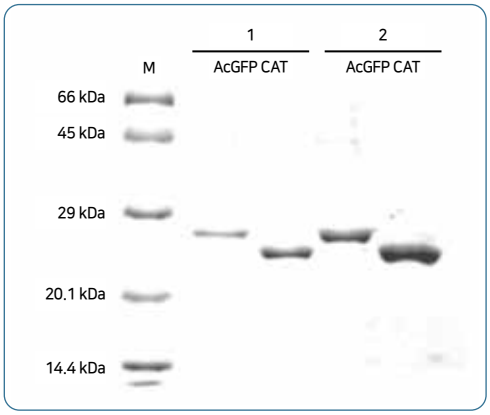
▶ Experimental Data

Template DNA was prepared using *ExiProgen*™ ProXpress PCR Template Kit. It was then used for protein synthesis using *ExiProgen*™ and *ExiProgen*™ EC Protein Synthesis Kit [Cat. No. K-7300]. Results show that protein yields were affected by DNA purity.

Group	Sample	$A_{260/280}$	$A_{260/230}$
1	AcGFP	1.82	0.28
	CAT	1.76	0.95
2	AcGFP	1.87	1.62
	CAT	1.87	1.51

- Amount of template DNA used: Each PCR product 500 ng
- Protocol No.: 902
- Sampling: Total 20 µl (Purification sample 15 µl + 4X loading dye 5 µl) of sample is boiled at 95°C for 5 min
- SDS-PAGE gel electrophoresis: Load 10 µl of boiled sample onto each well [10 × 8 (cm), 10 wells]

The resulting gel electrophoresis is as shown below:



M: Protein Size Marker
1: Sample group 1
2: Sample group 2

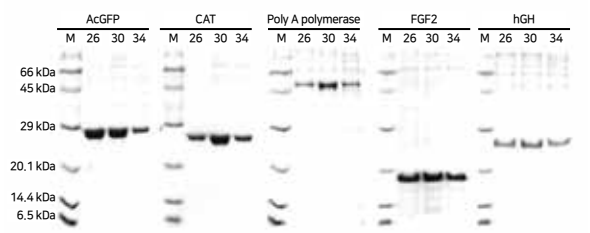
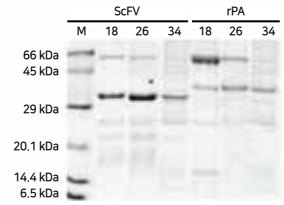
From the gel electrophoresis result above, it is evident that for the same protein and at the same condition, higher template DNA purity gives higher protein yield.

3.3. Effect of Reaction Temperature on protein expression

When cell-free protein expression is performed using *ExiProgen*™, the incubation temperature can affect the final amount of product as each protein has its optimal temperature for expression.

▶ Experimental Data

In this experiment, we observed the effect of reaction temperature on expression of AcGFP (Aequorea victoria Green Fluorescence Protein), CAT (Chloramphenicol Acetyl Transferase), FGF2 (Fibroblast Growth Factor 2), hGH (human Growth Hormone), Poly A polymerase, and proteins containing disulfide bond: ScFV (Single chain variable fragment) and rPA (recombinant Plasminogen Activator).

Description / Condition		
Protein type	Normal Proteins	Disulfide bonded proteins
Kit	*ExiProgen*™ EC-Maxi Protein Synthesis Kit	*ExiProgen*™ EC-Disulfide Protein Synthesis Kit
Template DNA	pBIVT-AcGFP (6 μg), pBIVT-CAT (6 μg), pBIVT-FGF2 (1.2 μg), pBIVT-hGH (1.2 μg), pET15b(+)-Poly A polymerase (6 μg), ScFV (1 μg), rPA (2 μg)	
Protein Expression Condition	Sample: AcGFP, CAT, FGF2, hGH, Poly A polymerase Reaction temperature: 26, 30, 34°C Protocol No.: 903	Sample: ScFV, rPA Reaction temperature: 18, 26, 34°C Protocol No.: 905
Protein Purification Condition	Bead: Ni-NTA magnetic bead 50 mg Final sample: 0.25 ml	
Result	Sampling: Total 20 μl (Purification sample 5 μl + 4X loading dye 5 μl + Sterile distilled water 5 μl) → Boil at, 95°C, 5 min SDS-PAGE gel electrophoresis: Load 10 μl of boiled sample in each well [10 × 8 (cm), 10 wells]	
	① Normal proteins  	M : Protein Size Marker 26: 26 °C setting 30: 30 °C setting 34: 34 °C setting
	② Disulfide bonded proteins  	M : Protein Size Marker 18: 18 °C setting 26: 26 °C setting 34: 34 °C setting

3.4. Screening for Protein Expression Optimization

AccuRapid™ Cell-Free Protein Expression Kit can be used to confirm if the target protein is expressible or not, in a fast and convenient way. Moreover, it can be used to find the optimized expression condition, i.e., addition of chaperones or chemicals that aid protein expression efficiency, or dispensing different amount of template DNA, onto different wells and identifying the condition that gave the best yield.

AccuRapid™ Cell-Free Protein Expression Kit [Cat. No. K-7250]

This kit is used to manually synthesize protein for small amounts and up to 24 samples can be processed at one time. It includes *E. coli* extract and Master mix to synthesize about 20 μg of protein of interest within 3 hours. *E. coli* extract contains T7 RNA polymerase and Master mix contains amino acids, rNTPs, energy source and other components required for protein expression.

▶ Features and Benefits

- **Rapid production**
  Protein expression from template DNA (Plasmid or PCR product) within only 3 hours.
- **Convenience**
  All the elements needed for the expression included in the kit.
- **Diversified range**
  Wide range of synthesizable protein sizes (10-150 kDa) from various types of DNA (plasmid or PCR product).
- **Advanced expression technologies**
  Expression of proteins (cell-toxic proteins, antibodies, membrane proteins, viral proteins, etc.) difficult to be done in the existing *in vivo* technology is possible.
- **Comprehensiveness**
  Compatible with fully automatic protein synthesis instrument, *ExiProgen*™ EC Protein Synthesis Kit series.

Not only the features introduced above, but the kit also has several other important uses:

Since,

i. Only a small amount (45 μl) of reaction volume is needed.
ii. 24 samples can be treated at one time.
iii. Each sample can have different conditions, like DNA concentration, protein types, concentration of other components needed for protein expression, etc.
iv. The whole expression process takes less than 3 hours.

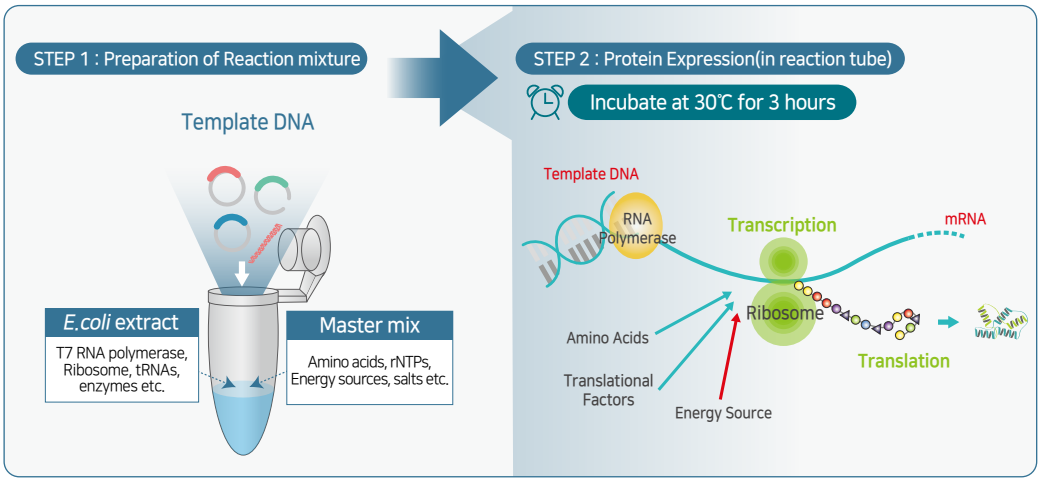
The kit is made highly useful for screening, to:

i. Test if a certain protein is expressible or not, prior to actual mass synthesis.
ii. Identify the optimal DNA concentration for protein expression.
iii. Optimize variable conditions for protein synthesis, etc.

Screening to confirm protein expression of various proteins

▶ Procedure and Principle

1. Mix the prepared template DNA (plasmid or PCR product) with the *E. coli* extract and Master mix included in the kit.
2. Incubate the reaction mixture at 30°C for 3 hours.



Although the synthesized protein will generally be detectable with Coomassie blue staining, Western Blot or fluorescence can also be used for detection.

▶ Experimental Data

1. Expression of various proteins

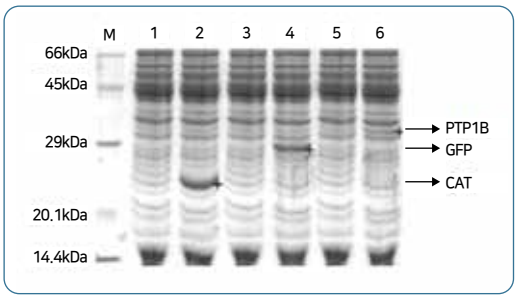


Figure 1. Various protein expression data (SDS-PAGE with Coomassie Brilliant Blue staining).

M: Protein Size Marker

Lane 1, 3, 5: Negative control (No-DNA)

Lane 2: CAT (Chloramphenicol acetyl transferase)

Lane 4: GFP (Green fluorescence protein)

Lane 6: PTP1B (Protein tyrosine phosphatase 1B)

Screening to optimize template DNA concentration

Using *ExiProgen*™ EC Protein Synthesis Kit series without knowing the optimal template DNA concentration might affect the protein yield. *AccuRapid*™ Cell-Free Protein Expression Kit can be used to determine the optimal amount of template DNA at small scale prior to large-scale protein production. The optimal concentration known can then be used for producing protein in large scale, using *ExiProgen*™ Kit series to obtain maximum yield.

▶ Procedure

1. **Prepare multiple tubes of different DNA concentrations:** 25, 50, 100, 200, 300, 400 (ng/μl)
2. **Prepare reaction mixture:**
   - Reaction volume : 45 μl
   - Sample DNA : Add 2 μl of each DNA concentration prepared
     → This makes a final DNA amount: 50, 100, 200, 400, 600, 800 ng
3. **Incubate the reaction mixture at 30°C for 3 hours**
4. **Analyze the samples with SDS-PAGE.**
   - To confirm the optimal concentration of template DNA for the highest yield.

▶ Experimental Data

1. **Screening optimal DNA concentration of Template DNA**

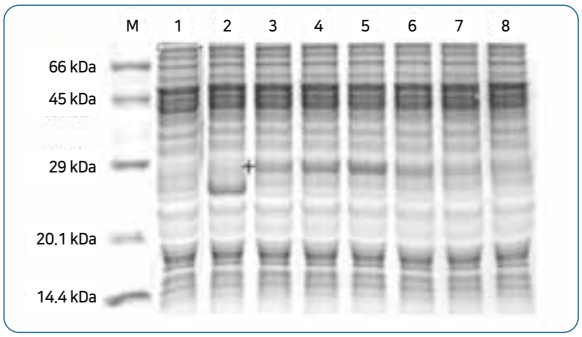


Figure 3. Determination of the optimal amount of template DNA for the expression of pBIVT-GFP.
(SDS-PAGE with Coomassie Brilliant Blue staining)
M: Protein Size Marker
Lane 1: Negative Control (No-DNA)
Lane 2: CAT (Chloramphenicol acetyl transferase)
Lane 3-9: pBIVT-GFP [50, 100, 200, 400, 600, 800 (ng)]

3.5. Batch expression vs. Continuous expression

When expressing protein in a cell-free manner, the limited energy source also limits the yield of the final expressed protein. If energy is constantly provided into a system, however, the amount of expressed protein can be maximized.

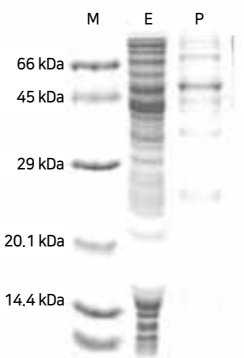
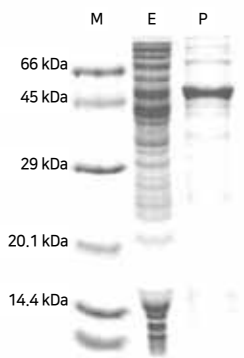
▶ Experimental Data

A synthesis of Poly A polymerase, known to be difficult to synthesize through cell culture, was done in two different ways of cell-free expression:

1) batch expression with limited source of energy.

2) continuous cell-free expression with unlimited source of energy.

As a conclusion, it is confirmed that the continuous supply of energy source gives greater amount of final expressed protein.

<table>
<tr><th colspan="4">Description / Condition</th></tr>
<tr><td>Feeding type</td><td colspan="2">(1) Batch type</td><td>(2) Continuous type</td></tr>
<tr><td>Kit</td><td colspan="3">AccuRapid™ Midi Protein Expression Kit</td></tr>
<tr><td>Template DNA</td><td colspan="3">pET15b(+)-Poly A polymerase (6 μg)</td></tr>
<tr><td>Protein Expression Condition</td><td colspan="2">Reaction mixture: 750 μl
Temperature: 26℃
Incubation time: 3 hrs
Equipment: Water bath</td><td>Reaction mixture: 750 μl
Temperature: 26℃
Incubation time: 22 hrs
Equipment: Hybridization incubator
Feeding buffer: 100 ml
Cut-off size of dialysis membrane: 6-8 kDa</td></tr>
<tr><td>Protein Purification Condition</td><td colspan="3">Bead: Ni-NTA magnetic bead 50 mg
Final sample: 0.25 ml</td></tr>
<tr><td rowspan="3">Result</td><td colspan="3">Sampling: Total 20 μl (Purification sample 15 μl + 4X loading dye 5 μl) → Boil at 95℃, 5 min
SDS-PAGE gel electrophoresis: Load 10 μl of boiled sample in each well
[10 × 8 (cm), 10 wells]</td></tr>
<tr><td>(1) Batch type</td><td>(2) Continuous type</td><td rowspan="2">M: Protein Size Marker
E: Expression sample
P: Purification sample</td></tr>
<tr><td> 
</td><td> 
</td></tr>
</table>

3.6. Improvement of Protein Expression Level

BIONEER has developed **Protein Expression Optimization Kit**. The kit screens 6 different types of expression enhanced tags to identify the most efficient tag, giving a possible solution to the problem of low protein expression level.

ExiProgen™ Protein Expression Optimization Kit [Cat. No. K-7410]

This kit is used to generate linear template DNA that contains six different types of tag sequences, which can be used to screen various tags for maximizing the target protein expression. This enables expressing proteins that were once difficult to express using the standard *ExiProgen*™ protein synthesis instrument.

Furthermore, each of the template DNA contains TEV cleavage sites, allowing removal of tags after the protein synthesis if needed.

▶ Features and Benefits

- **Rapid production**
  Acquisition of template DNA is done through PCR only but cloning is not required.
- **Convenience**
  Contains all the components essential for generating maximum of 30 different types of template DNA (1st primer set must be separately ordered for first PCR).
- **Minimized PCR error**
  Includes high-fidelity premix having high accuracy and precision, *AccuPower® ProFi Taq* PCR PreMix, to minimize the error rate during PCR.

▶ Experimental Data

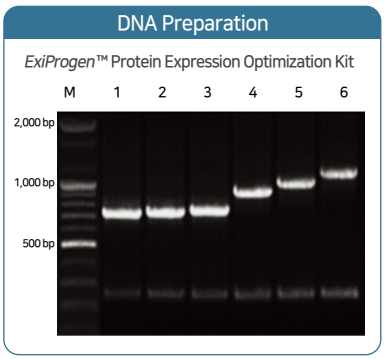


Figure 1. Template DNA construction using the control DNA.
Each linear DNA was generated by using *ExiProgen*™ Protein Expression Optimization Kit and 2nd PCR product samples are loaded on 1% agarose gel. The length of the control DNA is 462 bp.
M: DNA Ladder
Lane 1-6: Tag 1-6 inserted samples

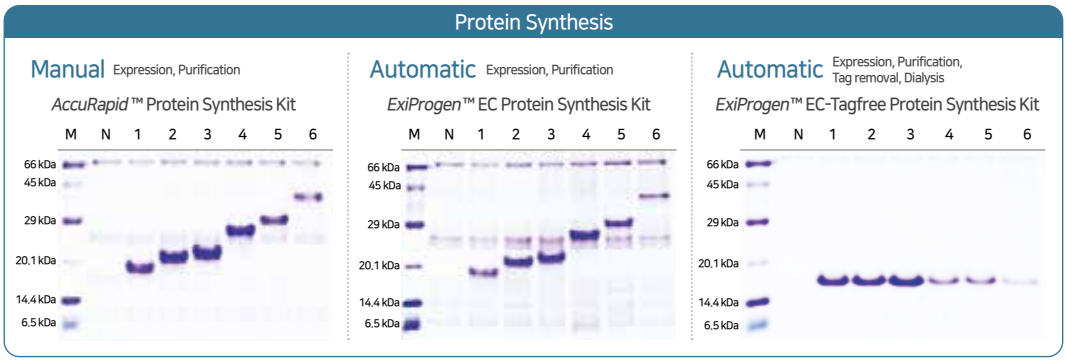


Figure 2. SDS-PAGE result of synthesized protein of the control DNA.

The linear DNA produced by using *ExiProgen*™ Protein Expression Optimization Kit was used as template DNA for protein synthesis with our various protein synthesis kits. The 22.5 µl of the purified proteins were loaded on 12% SDS-PAGE gel synthesized using 1 µg of DNA. The molecular weight of the control protein is 18 kDa.

M: Protein Size Marker

N: Non-tagged samples

Lane 1-6: Tag 1-6 inserted samples

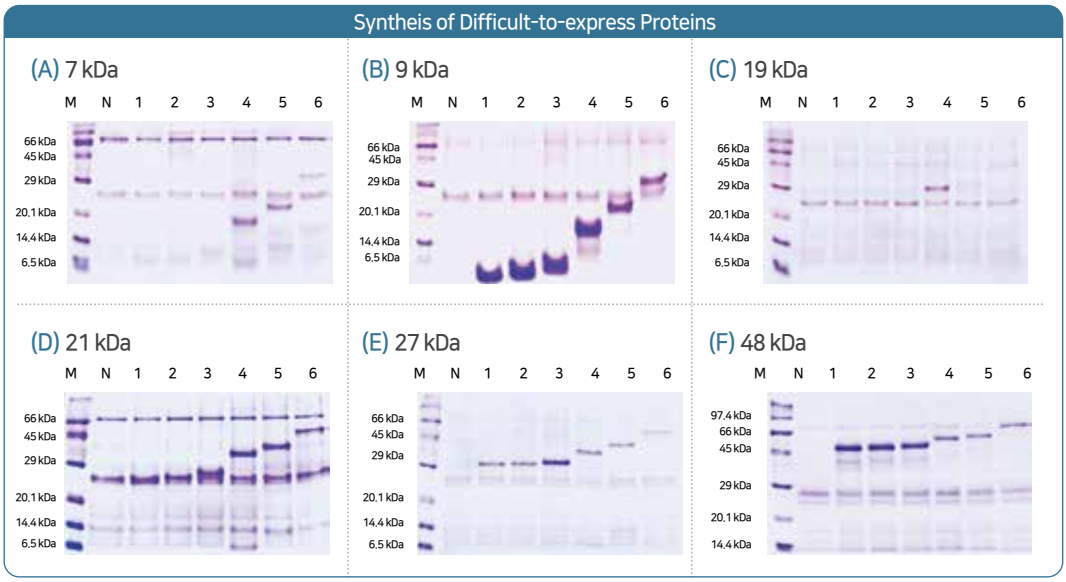


Figure 3. SDS-PAGE result of synthesized proteins of various sizes of difficult-to-express proteins.

Difficult-to-express proteins were synthesized using the template DNAs generated by the *ExiProgen*™ Protein Expression Optimization Kit and *ExiProgen*™ EC Protein Synthesis Kit.

M: Protein Size Marker

N: Non-tagged samples

Lane 1-6: Tag 1-6 inserted sample

3.7. Exemplary Cases of Protein Synthesis

Toxic protein synthesis using *ExiProgen*™

Cell-based protein production method is not easy to apply to proteins that have cytotoxic effects on cells. Those proteins may harm the cells during the augmentation process of their expression vector. *ExiProgen*™ will be a solution to proteins that are cytotoxic because protein expression is performed in cell-free system.

▶ Experimental Data

For cytotoxic gene samples, DNase I and Protease K were used. *ExiProgen*™ ProXpress PCR Template Kit was used to obtain Template DNAs for each of the two proteins. The template DNAs were then used for protein synthesis by the instrument *ExiProgen*™. The experimental steps are as follows:

- Step 1: Gene synthesis

  Genes were synthesized using BIONEER's Gene synthesis service and cloned into an expression vector.

- Step 2: Template DNA preparation

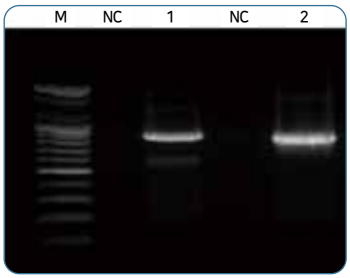


Figure 1. Template DNA construction for toxic protein synthesis.
pT-DNase I and pT-Proteinase K were prepared using *ExiProgen*™ ProXpress PCR Template Kit [Cat. No. K-7400].
M: 100 bp DNA Ladder [Cat. No. D-1030]
NC: Negative control (No-template)
1: pT-DNase I
2: pT-Proteinase K

- Step 3: Confirmation of protein expression

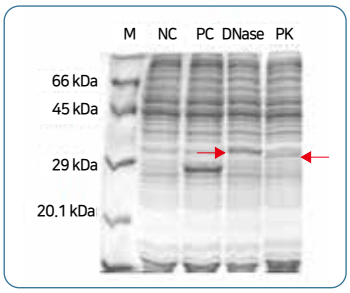


Figure 2. Confirmation of toxic protein expression in small scale.
Using *AccuRapid*™ Cell-Free Protein Expression Kit [Cat. No. K-7250], 10 ng of each of positive control (AcGFP), DNase I and Proteinase K proteins were manually expressed.
- Reaction condition: Incubation at 30°C for 3 hours.
- Sampling: Total 20 µl (Expression sample 5 µl + D.W. 10 µl + 4X loading dye 5 µl) → Boil at 95°C, 5 min
- SDS-PAGE gel electrophoresis: Load 5 µl of boiled sample in each well [10 × 8 (cm), 10 wells].

M: Protein Size Marker
NC: Negative control (No-DNA)
PC: Positive control (AcGFP)
DNase: DNase I
PK: Proteinase K

Step 4: Protein synthesis

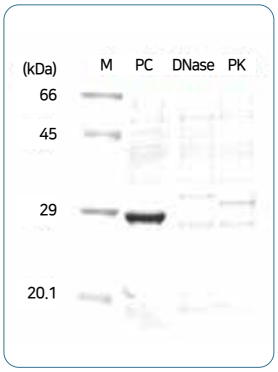


Figure 3. Confirmation of protein synthesis through SDS-PAGE.
Using *ExiProgen*™ EC Protein Synthesis Kit [Cat. No. K-7300], 500 ng of each of positive control (AcGFP), DNase I and Proteinase K proteins were automatically expressed.
- Sampling: Total 20 µl (Purification sample 15 µl + 4X loading dye 5 µl) → Boil at 95°C, 5 min
- SDS-PAGE gel electrophoresis: Load 10 µl of boiled sample in each well [10 × 8 (cm), 10 wells]

M: Protein Size Marker
PC: Positive control (AcGFP)
DNase: DNase I
PK: Proteinase K

Step 5: Checking protein activity

(A) DNase I

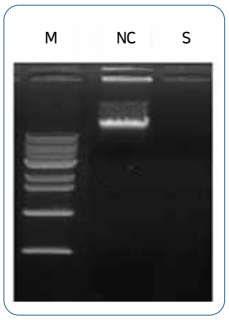


Figure 4. Lambda DNA was degraded by DNase I synthesized with *ExiProgen*™.
(All sample were incubated at 37°C for 3 hours.)

M: 1 kb DNA Ladder [Cat. No. D-1040]
NC: Negative control (No DNase I treated)
S: DNase I treated sample

(B) Proteinase K

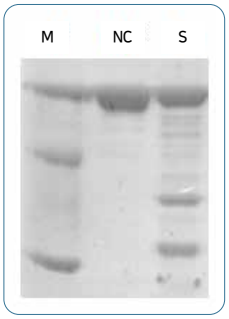


Figure 5. BSA protein was degraded by Proteinase K synthesized with *ExiProgen*™.
(All samples were incubated at 37°C for 16 hours.)

M: Protein Size Marker
NC: Negative control (No Proteinase K treated)
S: Proteinase K treated sample

Membrane protein synthesis using *ExiProgen*™

Membrane protein is generally hard to get expressed in *E. coli* system, either *in vivo* or *in vitro* expression system, so detergent addition is one of the solutions for successful membrane protein synthesis. *ExiProgen*™ protein synthesis kit provides the easiest solution for synthesizing membrane proteins simply by adding detergent to the samples.

▶ Experimental Data

In this experiment, protein expression vector was constructed by gene synthesis of FLAP (5-Lipoxygenase Activating Protein, containing four trans-membrane domains) and bR (bacteriorhodopsin, containing seven trans-membrane domains) with pET32a(+) cloning. This protein expression vector was used as template DNA for protein synthesis using *ExiProgen*™.

◦ Step 1: Gene synthesis

E. coli codon optimized genes were synthesized by using BIONEER's Gene synthesis service.

◦ Step 2: Template DNA preparation using Cloning method

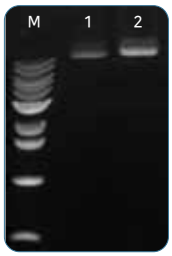


Figure 1. pT-FLAP and pT-bR inserts were cloned into pET32(+) vector DNA.

Through the cloning process, template DNA of pET32a(+)-FLAP and pET32a(+)-bR were produced.

Vector DNA: pET32a(+)
Insert DNA: pT-FLAP, pT-bR
Competent cell: DH5α
M: 100 bp DNA Ladder [Cat. No. D-1030]
Lane 1: pET32a(+)-FLAP
Lane 2: pET32a(+)-bR

◦ Step 3: Protein synthesis

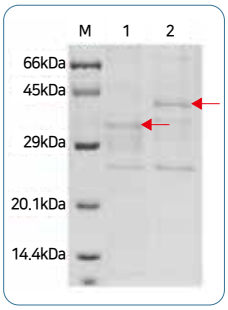


Figure 2. Synthesized protein was confirmed through SDS-PAGE.

ExiProgen™ EC Protein Synthesis Kit [Cat. No. K-7300] was used to synthesize protein from template DNA, and detergent was added to collect protein in their native structure.

- Template DNA: pET32a(+)-FLAP (3 μg): pET32a(+)-bR (6 μg)
- Detergent addition: Add expressed protein solution and purification buffer to final 0.5% BriJ35
- Sampling: Total 20 μl (Purification sample 15 μl + 4X loading dye 5 μl) → Boil at 95℃, 5 min
- SDS-PAGE gel running: Load 10 μl of boiled sample in each well [10 × 8 (cm), 10 wells]

M: Protein Size Marker
Lane 1: FLAP (35 kDa)
Lane 2: bR (43 kDa)

Troubleshooting guide

Please refer to the list below for some of the commonly asked questions and solutions if you encounter problems while using nucleic acid extraction products. The solutions listed below are only general suggestions and might not address all problems. For problems related to specific proteins, further optimization may be required.

1. No protein synthesis, including positive control protein.

Cause	Solution
Nuclease (DNase, RNase) contamination	When performing the experiment, wear gloves and use DNase- and RNase-free pipette tips.
Pipetting error or missing reagents	Calibrate your pipettes and make sure you add correct amount of reagents at all times. Make sure to add the correct amount of Positive control DNA and ensure that all components are installed correctly.
Storage condition of reagent	Store all reagents and components of the kit at the recommended temperatures. In particular, avoid repeated freeze-thaw cycles of *E. coli* extract.

2. Positive control protein is synthesized but target protein is not synthesized.

Cause	Solution
Sequences of template DNA	If there is a mutation in the ORF (Open Reading Frame) of the template DNA, translation may be interrupted, so please check the ORF sequence.
Structures of template DNA	Confirm that the structures of the T7 promoter, T7 terminator, and histidine tag are correctly positioned in the template DNA.
Contamination of template DNA	If DNA is degraded by nuclease contamination during preparation of template DNA, protein cannot be synthesized. It is recommended to use template DNA obtained using nuclease-free elution buffer.
Low expression efficiency	*ExiProgen*™ Protein Expression Optimization Kit can be applied to increase protein expression efficiency.

3. Low yield of the target protein

Cause	Solution
Purity of template DNA	If the purity of template DNA is low, the protein may not be synthesized. It is recommended to use DNA purity as follows: $A_{260/280}$: 1.7-2.0 and $A_{260/230}$: >1.5.
Amount of template DNA	There may be differences in the amount of synthesized protein depending on the amount of added template DNA. To increase the protein expression yield, optimize DNA concentration through expression rate screening test* prior to the experiment. * Refer to User Guide - Appendix B of *ExiProgen*™ EC Protein Synthesis Kit [Cat. No. K-7300]
Unoptimized template DNA for *E. coli* codons	If the sequences of the template DNA are not optimized for *E. coli* codons, protein expression may not occur or the expression level may be low. It is recommended to use codon-optimized DNA.
The position of His-tag	Depending on the location of the histidine tag, the expression of the target protein may be inhibited. Or, it might not affect protein expression but purification, as the His-tag is not exposed to the outside during the formation of the tertiary structure. In this case, change the position of the tag.

4. Low solubility or activity of the target protein

Cause	Solution
Proteins that require post-translational modification	In the cell-free protein synthesis system using *E. coli* extract, it is impossible to synthesize proteins that require post-translational modifications such as glycosylation and phosphorylation. Therefore, use eukaryotic protein expression system for protein expression, and then use *ExiProgen*™ only for purification.
Additional elements required for protein activity	If the synthesized protein requires specific elements to exhibit activity, the activity should be confirmed after adding these elements to the final purified protein solution.
Aggregation due to low solubility of proteins	If the protein expression temperature is lowered or the chaperone protein is added to help fold the protein, the solubility may be improved (Frydman, J., 2001)*

* Frydman J (2001) Folding of newly translated proteins *in vivo*: the role of molecular chaperones. *Annu Rev Biochem*, 70:603-647.

Related Products for Manual type Cell-Free Protein Expression, Synthesis and Purification

Protein Synthesis and Expression	
Cat. No.	**Product Description**
K-7250	*AccuRapid*™ Cell-Free Protein Expression Kit
Manual type protein expression kit including *E. coli* extract and Master mix. About 300 µg of protein is synthesized in a 1 ml reaction volume, reaction volume is 45 µl, and 24 reactions are possible at one time.	
K-7260	*AccuRapid*™ Midi Protein Expression Kit
Manual type protein expression kit, including *E. coli* extract and Master mix. About 300 µg of protein is synthesized in a 1 ml reaction volume, reaction volume is 1 ml, and 5 reactions are possible at one time.	
K-7270	*AccuRapid*™ Maxi Protein Expression Kit
Manual type protein expression kit, including *E. coli* extract and Master mix. About 300 µg of protein is synthesized in a 1 ml reaction volume, reaction volume is 10 ml, and only 1 reaction is possible at one time.	
K-7280	*AccuRapid*™ Protein Synthesis Kit
Manual type protein expression and purification kit, including *E. coli* extract, Master mix and Ni-NTA magnetic beads. Reaction volume is 750 µl, about 100 µg of protein is synthesized per reaction, and 5 reactions are possible at one time.	

Protein Purification	
Cat. No.	**Product Description**
K-7200	*MagListo*™ His-tagged Protein Purification Kit
Manual type protein purification kit (using magnetic separation), including Ni-NTA magnetic nanobeads (average 400 nm in diameter) and Nd magnet. About 3-4 mg of 6x His-tagged proteins can be bound onto about 1 ml of magnetic nanobeads.	
K-7210/20/30	*MagListo*™ Protein G, Protein A and Protein L Kit
Manual type antibody purification kit (using magnetic separation), including protein G/A/L magnetic nanobeads, respectively. Coated with high purity protein G/A/L, specific recognition of antibodies and purification of about 800 µg up to 1.2 mg Human IgG per 1 ml of bead solution is possible.	

Ordering Information

Cat. No.	Product Description
Automatic Protein Expression / Synthesis Kits	
K-7300~2	*ExiProgen*™ EC Protein Synthesis Kit (16/32/96 reactions)
K-7310	*ExiProgen*™ EC-Maxi Protein Synthesis Kit, 8 reactions
K-7320	*ExiProgen*™ EC-Tagfree Protein Synthesis Kit, 8 reactions
K-7330	*ExiProgen*™ EC-Disulfide Protein Synthesis Kit, 8 reactions
K-7340	*ExiProgen*™ EC-Bulk Protein Synthesis Kit, 1 reaction
Automatic Protein Purification Kits	
K-7220~1	*ExiProgen*™ His-tagged Protein Purification Kit (16/32 reactions)
K-7240	*ExiProgen*™ Dialysis Kit
KA-3001	*ExiProgen*™ Consumable SET - can be used with *MagListo*™ Protein G/A/L Kit [Cat. No. K-7710/20/30]

Related Products

Cat. No.	Product Description
Manual Protein Expression / Synthesis Kits	
K-7250	*AccuRapid*™ Cell-Free Protein Expression Kit
K-7260	*AccuRapid*™ Midi Protein Expression Kit
K-7270	*AccuRapid*™ Maxi Protein Expression Kit
K-7280	*AccuRapid*™ Protein Synthesis Kit
Manual Protein Purification Kits	
K-7200	*MagListo*™ His-tagged Protein Purification Kit
K-7710	*MagListo*™ Protein G Kit
K-7720	*MagListo*™ Protein A Kit
K-7730	*MagListo*™ Protein L Kit
Magnetic Separation Rack	
TM-1000	*MagListo*™-8Ch Magnetic Separation Rack
TM-1010	*MagListo*™-2 Magnetic Separation Rack
TM-1011	*MagListo*™-2-12h Magnetic Separation Rack
TM-1020	*MagListo*™-15 Magnetic Separation Rack
TM-1030	*MagListo*™-50 Magnetic Separation Rack
Ladder	
D-1030	*AccuLadder*™ 100 bp DNA Ladder
D-1040	*AccuLadder*™ 1kb DNA Ladder
D-2030	*AccuLadder*™ 3-color Prestained Protein Size Marker (Broad)
Buffer	
C-9100	Agarose, 100 g
C-9002	5X TBE, 1 gal
C-9004	50X TAE, 500 ml
C-9027	10% Sodium Dodecyl Sulfate (SDS), 500 ml
C-9029	6X Agarose Gel Loading Buffer, 2 ml

Service information

Cat. No.	Service Description
S-2500	Standard Protein Synthesis Service
S-2600	One-day Protein Synthesis Service
Custom	Gene to Protein Synthesis Custom Order

* For Custom Services, please inquire to order, or enter BIONEER's website to order.

Email address for inquiry: proteinorder@bioneer.co.kr **BIONEER's website:** www.bioneer.com

Tel. 1588-9788 for Customer Service

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