J Protein Expression and Purification

Automated Protein Expression and Purification: *ExiProgen*™
Manual Protein Expression and Purification: *AccuRapid*™ & *MagListo*™
Preparation of Template DNA for Protein Expression
Protein Service

01

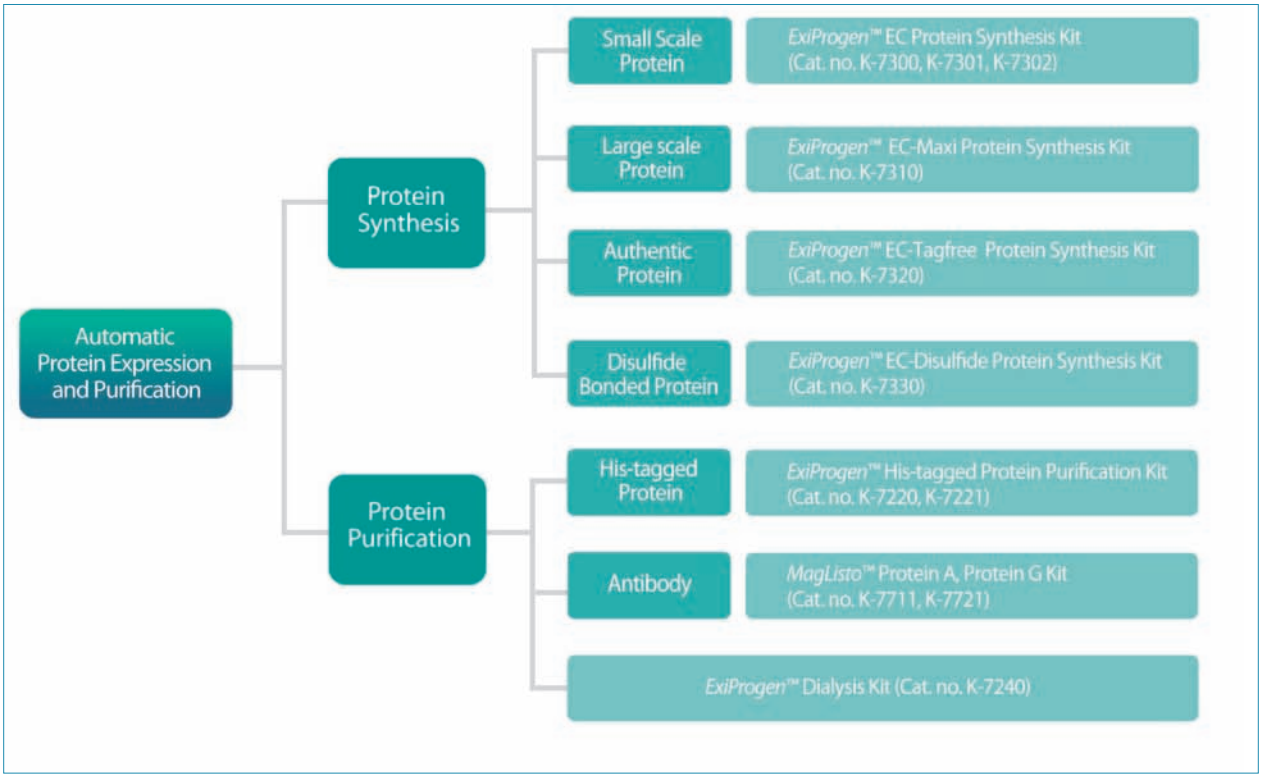
Automated Protein Expression and Purification: *ExiProgen*™

Overview

Bioneer currently has a number of cell-free protein synthesis kits based on *E. coli* expression system. *ExiProgen*™'s cell-free expression system uses T7 RNA polymerase. Thus, any gene of interest under the control of T7 promoter can be expressed using *ExiProgen*™.

Among the products, EC and EC-Maxi kits produce about 100 µg and 500 µg of protein per reaction, respectively, in which no or one disulfide bonds are present. Also, EC-Disulfide is available for proteins containing more than one disulfide bonds. Bioneer's specialized tag-free protein synthesis kits can be used to produce highly pure proteins without histidine-tag.

ExiProgen™ Guide Book for principle and detailed contents about *ExiProgen*™ instrument and its related products is available. After fill in application form at Bioneer homepage (www.bioneer.com), please download the *ExiProgen*™ Guide Book for free.



ExiProgen™ EC Protein Synthesis Kit

Checking up to 16 different protein syntheses at a time

Description

This kit is for automated protein synthesis using *ExiProgen*™, and consisted of Expression Cartridge and Purification Cartridge. By adding your template DNA into a well in the Expression Cartridge and installing the two cartriges into *ExiProgen*™, your target protein is automatically expressed and purified. Using protocol no. 902 (approx. 6 hours), 16 different pure proteins can be obtained without tedious manual processing for each individual protein. Proteins can be expressed and purified with high reproducibility and with minimal variations due to *ExiProgen*™'s automated process and pre-optimized protocols.

Features and Benefits

- Fully Automatic: DNA-in-Protein-out
  Input 1~10 µg of template DNA and load the cartridges into *ExiProgen*™ for high-purity proteins within 6 hours.
- High Performance
  The system allows for synthesis of proteins that are toxic to *in vivo* expression systems.
- High Purity and High Yield
  Each reaction well will yield up to 100 µg protein that is over 90% pure.
- Reproducibility
  The only hands-on step is the addition of template DNA, maximizing reproducibility.

Application

This kit can be used in protein research and development including enzyme for research and business. In particularly, it can be applied in an analysis of various mutant screening.

Experimental Data

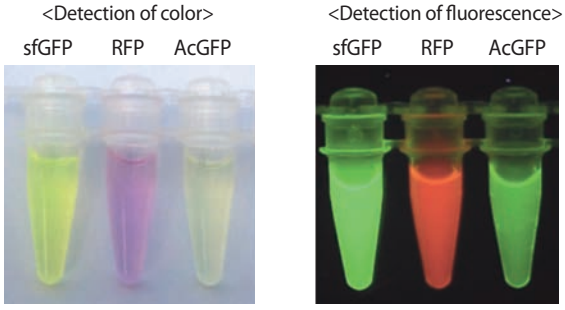


Figure 1. Detection of fluorescence emitted from fluorescent proteins synthesized with *ExiProgen*™.

This result indicates that these synthesized sfGFP, RFP, and AcGFP are functionally active.
Left panel: Color of each protein elution samples detected with naked eyes.
Right panel: Fluorescence from protein elution samples detected with UV illuminator.

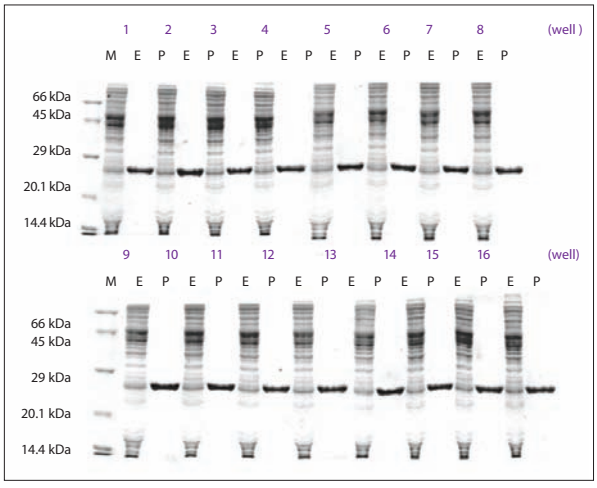


Figure 2. Expression and purification of CAT (Chloramphenicol acetyltransferase). Reproducibility is seen in all 16 wells with no detectable variation between wells.

Lane 1~16: Number of the well
M: *AccuLadder*™ Protein Size Marker (Low)
E: Expression sample
P: Purification sample

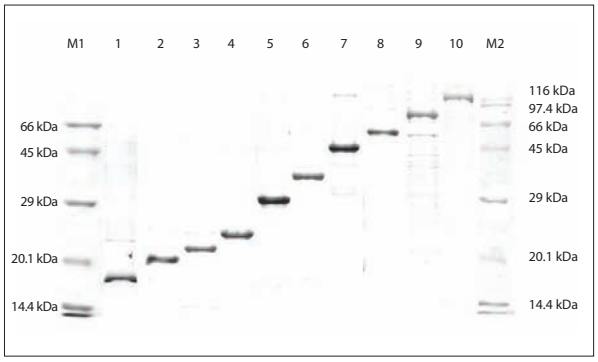


Figure 3. PAGE data of various proteins synthesized from various templates (Plasmid DNA-10~120 kDa, PCR product-10~60 kDa).

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 total sample.

M1: *AccuLadder*™ Protein Size Marker (Low)

1: CalmL3 (17.5 kDa), 2: RNase H (20 kDa), 3: DUSP 3 (22 kDa)

4: CAT (24 kDa), 5: AcGFP (29 kDa), 6: EF-Ts (34 kDa)

7: VF (45 kDa), 8: Poly A polymerase (50 kDa)

9: *M-MLV* RTase (75 kDa), 10: BM3 (117 kDa)

M2: *AccuLadder*™ Protein Size Marker (Broad)

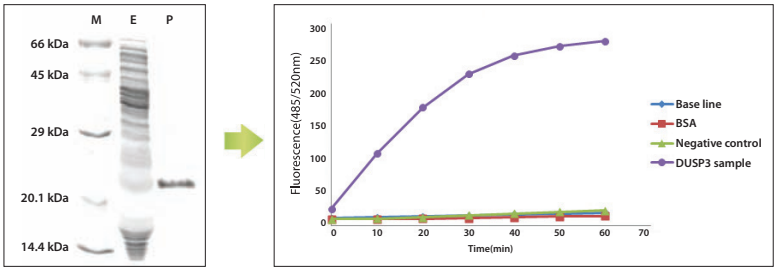


Figure 4. PAGE & enzyme activity data of DUSP3 (Dual Specificity Phosphatase 3).

Left panel: Expression and purification of DUSP3.

M: *AccuLadder*™ Protein Size Marker (Low)

E: Expression sample, P: Purification sample

Right panel: DUSP3 synthesized with *ExiProgen*™ has an enzyme activity.

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

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

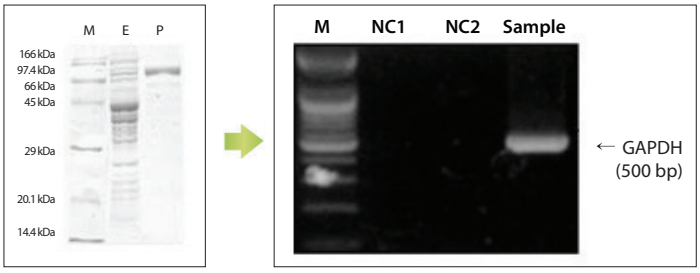


Figure 5. PAGE & enzyme activity data of *M-MLV* RTase.

Left panel: Expression and purification of *M-MLV* RTase.

M: *AccuLadder*™ Protein Size Marker (Broad)

E: Expression sample, P: Purification sample

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

Lane M: 100 bp DNA Ladder

Lane NC1: Negative control 1 (No-RNA)

Lane NC2: Negative control 2 (No-enzyme)

Lane Sample: 10 ng Human total RNA from HeLa cell

Ordering Information

Cat. no.	Product Description
K-7300	*ExiProgen*™ EC Protein Synthesis Kit, 16 rxns
K-7301	*ExiProgen*™ EC Protein Synthesis Kit, 32 rxns
K-7302	*ExiProgen*™ EC Protein Synthesis Kit, 96 rxns
Related Product	
K-7400	*ExiProgen*™ ProXpress PCR Template Kit, 16 rxns
K-2631	*AccuPower® ProFi Taq* PCR PreMix, 96 tubes, 20 μl rxn/tube
K-7350	pBIVT Vectors Set-1
K-3035	*AccuPrep®* Gel Purification Kit, 200 rxns
K-3034	*AccuPrep®* PCR Purification Kit, 200 rxns
D-2020	*AccuLadder*™ Protein Size Marker (Low), 500 μl
D-2010	*AccuLadder*™ Protein Size Marker (Broad), 500 μl
A-5041	*ExiProgen*™, Instrument

01 *ExiProgen*™ EC-Maxi Protein Synthesis Kit

Obtaining over 500 μg of protein easily

Features and Benefits

- Fully Automated: DNA-in-Protein (in storage buffer)-out
  After simple addition of template DNA into a cartridge well, the target protein is synthesized and harvested.
- Parallel Processing
  1-8 different proteins can be synthesized in a single run.
- High Purity and High Yield
  Up to 500 μg of protein can be obtained per well with a purity of >90%.
- Free choice of protein storage buffer
  Homemade protein storage buffer can be used (10% glycerol in the buffer is a minimum requirement.).

Description

This kit is for automated large scale synthesis of a target protein using the patented SECF (Stepwise Exchange Cell-Free) protein synthesis technology, and the kit is consisted of Expression Cartridge and Purification Cartridge. After adding template DNA into the Expression Cartridge and installing the two cartridges into *ExiProgen*™, up to 500 μg of target protein can be automatically synthesized within a day. Using protocol no. 903 (approx. 25 hours), all processes including protein synthesis and dialysis will be performed in an automated fashion and the final protein harvested in a storage solution of choice.

Application

This kit can be used in research and development of proteins and enzymes.

Experimental Data

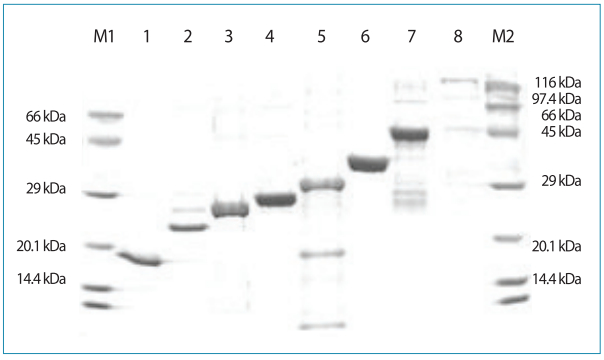


Figure 1. Synthesis of various proteins.

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

M1: *AccuLadder*™ Protein Size Marker (Low)

1: CalmL3 (17.5 kDa) 2: DUSP 3 (22 kDa) 3: CAT (24 kDa)

4: AcGFP (29 kDa) 5: RFP(30 kDa) 6: EF-Ts (34 kDa)

7: VF (45 kDa) 8: BM3 (117 kDa)

M2: *AccuLadder*™ Protein Size Marker (Broad)

Ordering Information

Cat. no.	Product Description
K-7310	*ExiProgen*™ EC-Maxi Protein Synthesis Kit, 8 rxns
Related Product	
K-7350	pBIVT Vectors Set-1
K-3030	*AccuPrep*® Plasmid Extraction Kit, 200 rxns
K-3034	*AccuPrep*® PCR Purification Kit, 200 rxns
D-2020	*AccuLadder*™ Protein Size Marker (Low), 500 μl
D-2010	*AccuLadder*™ Protein Size Marker (Broad), 500 μl
A-5041	*ExiProgen*™, Instrument

ExiProgen™ EC-Tagfree Protein Synthesis Kit

Obtaining His-tag removed proteins

Description

This kit is used for automated synthesis of a protein without his-tag sequence with high purity on *ExiProgen*™, and it is consisted of Expression Cartridge and Purification Cartridge. After adding template DNA into the Expression Cartridge and installing the two cartridges into *ExiProgen*™, the protein is synthesized with his-tag first. Then the his-tag is cleaved off, resulting in a pure protein without a his-tag sequence. Using protocol no. 904 (approx. 25 hours), all processes, including protein synthesis and dialysis, are performed in an automated manner and the final protein is harvested in any storage buffer of choice.

Features and Benefits

- Protein Synthesis without His-tag
  A target protein is produced in a fully automated way then its his-tag is cleaved from the final protein.
- Fully Automatic: DNA-in-Protein (in storage buffer)-out
  Purified target protein will be in any storage buffer of choice.
- Parallel Processing and High Purity
  1-8 different proteins can be synthesized in a single run with purity >95%.
- Free choice of protein storage buffer
  Homemade protein storage buffer can be used (10% glycerol in the buffer is a minimum requirement.).

Application

This kit is can be applied in X-ray crystallography, therapeutic protein development, and antigen expression for antibody development.

Experimental Data

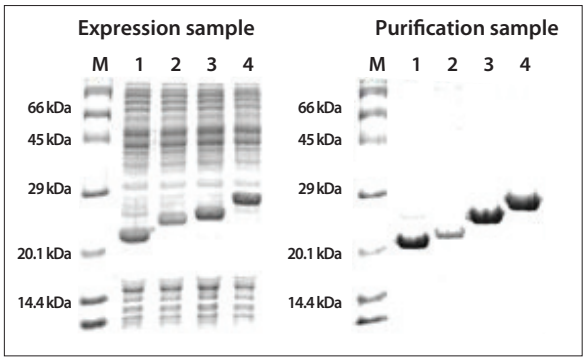


Figure 1. Expression and purification of various proteins.
M: *AccuLadder*™ Protein Size Marker (Low) (D-2020, Bioneer)
1: DUSP3 (22 kDa), 2: hGH (23 kDa), 3: CAT (24 kDa), 4: AcGFP (28 kDa)
Note) DUSP3: Dual specificity protein phosphatase 3, hGH: Human growth hormone

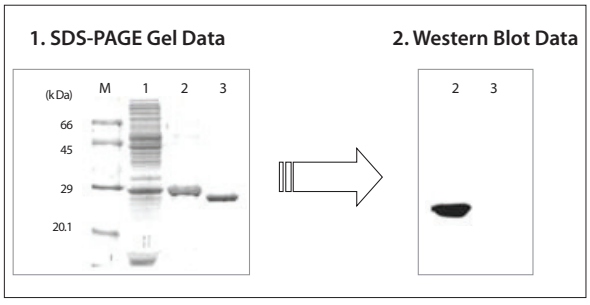


Figure 2. Data of SDS–PAGE and Western blot with His–tag antibody.
M: *AccuLadder*™ Protein Size Marker (Low)
1: Expression sample of pBIVT–TEV–AcGFP
2: His tag–TEV–AcGFP (His–tagged sample)
3: AcGFP (His–tag free sample)

Ordering Information

Cat. no.	Product Description
K-7320	*ExiProgen*™ EC-Tagfree Protein Synthesis Kit, 8 rxns
Related Product	
K-7350	pBIVT Vectors Set-1
K-3030	*AccuPrep*® Plasmid Extraction Kit, 200 rxns
K-3034	*AccuPrep*® PCR Purification Kit, 200 rxns
D-2020	*AccuLadder*™ Protein Size Marker (Low), 500 µl
D-2010	*AccuLadder*™ Protein Size Marker (Broad), 500 µl
A-5041	*ExiProgen*™, Instrument

ExiProgen™ EC-Disulfide Protein Synthesis Kit

Obtaining target protein with disulfide bond

Description

This kit is used for synthesizing a protein that contains disulfide bonds using *ExiProgen*™, and it is consisted of Expression Cartridge and Purification Cartridge. After adding the template DNA into the Expression Cartridge and installing the two cartridges into *ExiProgen*™, the protein that contains up to nine disulfide bonds is synthesized in a fully automated manner and more efficiently than manner of cell-based synthesis. Using protocol no. 905 (approx. 36 hours), all processes, including protein synthesis and dialysis, are performed in an automated manner and the final protein is harvested in any storage buffer of choice.

Features and Benefits

- Protein Synthesis with Disulfide Bond
  This kit is used to synthesize a protein that has two or more (1~9) disulfide bonds, providing its intact function.
- Fully Automatic: DNA-in-Protein (in storage buffer)-out
  Purified target protein is eluted in a storage buffer of choice.
- Free choice of protein storage buffer
  Homemade protein storage buffer can be used (10% glycerol in the buffer is a minimum requirement.).
- Parallel Processing and High Purity
  1-8 different proteins can be synthesized in a single run.

Application

This kit is can be used to produce pharmaceutical protein and ScFv for antibody screening.

Experimental Data

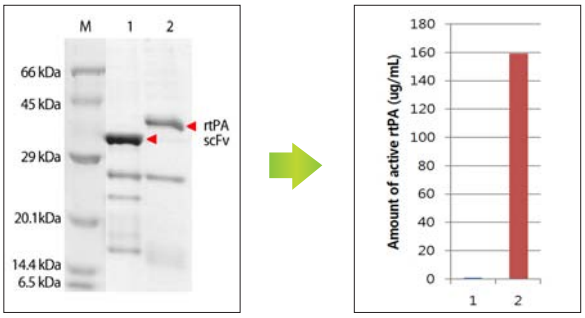


Figure 1. SDS- PAGE & enzyme activity data.
Left Pannel: Expression and purification of ScFV and rtPA in *ExiProgen*™
M: *AccuLadder*™ Protein Size Marker (Low)
1: ScFV containing 2 disulfide bonds
2: rtPA containing 9 disulfide bonds
* Amount of purification sample is 1/40 of total sample.
Right panel: rtPA synthesized has an enzyme activity.
1: Synthesis sample using *ExiProgen*™ EC-Maxi Protein Synthesis Kit
2: Synthesis sample using *ExiProgen*™ EC-Disulfide Protein Synthesis Kit
* rtPA activity was measured by incubating with chromogenic substrate S-2288.

Ordering Information

Cat. no.	Product Description
K-7330	*ExiProgen*™ EC-Disulfide Protein Synthesis Kit, 8 rxns
Related Product	
K-7350	pBIVT Vectors Set-1
K-3030	*AccuPrep*® Plasmid Extraction Kit, 200 rxns
K-3034	*AccuPrep*® PCR Purification Kit, 200 rxns
D-2020	*AccuLadder*™ Protein Size Marker (Low), 500 µl
D-2010	*AccuLadder*™ Protein Size Marker (Broad), 500 µl
A-5041	*ExiProgen*™, Instrument

01 *ExiProgen*™ His-tagged Protein Purification Kit

Purification of cell-free protein

Description

ExiProgen™ His-tagged Protein Purification Kit is used with *ExiProgen*™ for purification of a his-tagged target protein (protocol no. 901, approx. 2 hours) contained in *E. coli* lysate or cell-free protein synthesis reaction mixture. The kit contains Ni-NTA magnetic beads and all other reagents as a pre-filled cartridge. Ni-NTA magnetic beads are silica-coated magnetic particles with a functional group of Ni^{2+}-nitrilotriacetic acid, which possesses an affinity to a histidine tag.

Features and Benefits

- Availability of Various Sample
  This kit can be used to purify proteins expressed in *E. coli* cells and cell-free system.
- Pre-filled Buffer Cartridge System
  Pre-filled cartridge containing all reagents required for protein purification simplifies the process.
- Parallel Processing and High Purity
  Up to 8 different proteins can be purified at one time with >90% purity.

Application

This kit is can be used to purify his-tagged target protein from *E. coli* cell lysate including that expressed target protein.

Experimental Data

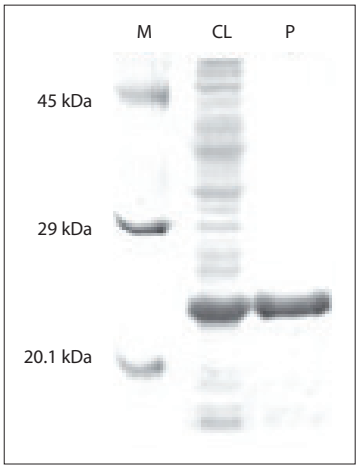


Figure 1. Purification of DUSP3 with *ExiProgen*™.
M: *AccuLadder*™ Protein Size Marker (Low)
CL: Sample of recombinant *E. coli* cell lysate (DUSP3 expression)
P: Sample of purification

Ordering Information

Cat. no.	Product Description
K-7220	*ExiProgen*™ His-Tagged Protein Purification Kit, 16 rxns
K-7221	*ExiProgen*™ His-Tagged Protein Purification Kit, 32 rxns
Related Product	
D-2020	*AccuLadder*™ Protein Size Marker (Low), 500 µl
D-2010	*AccuLadder*™ Protein Size Marker (Broad), 500 µl
A-5041	*ExiProgen*™, Instrument

ExiProgen™ Dialysis Kit[NEW]

Dialysis of protein

Description

This kit allows automatic dialysis of the protein using Bioneer's *ExiProgen*™. Aliquot desired buffer to the catridge provided with the kit and install it on *ExiProgen*™ with solution of target protein. Operate protocol then harvest the target protein in your own solution after 13 hours!

Features and Benefits

- Efficiency
  Buffer can be exchanged using small amount of buffer.
- Convenience
  Buffer exchange can be done at a low temperature without additional cooling system.
- Fully Automatic
  After loading the sample, all steps are automatic.
- Parallel Processing
  Buffers for sixteen different proteins can be exchanged simultaneously.

Application

Exchanging buffer for protein, buffer screening for optimizing protein work

Experimental Data

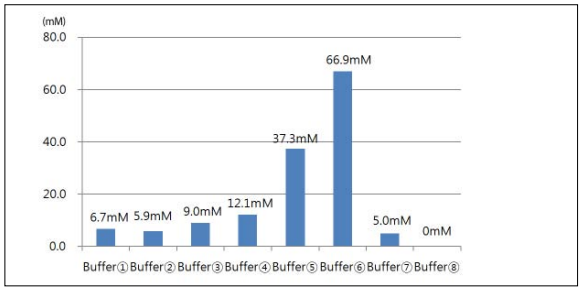


Figure 1. Comparison of efficiency according to different glycerol concentration.
After automatic dialysis, the final concentration of imidazole is measured by UV-spectrophotometer.
1: buffer 1 consisting of 50 mM Tris-Cl (pH7.6), 100 mM NaCl, 0.2 mM EDTA, 0.05% NaN3,
2: buffer 1 including 10% Glycerol,
3: buffer 1 including 20% Glycerol,
4: buffer 1 including 30% Glycerol,
5: buffer 1 including 40% Glycerol,
6: buffer 1 including 50% Glycerol,
7: PBS buffer ;
8: buffer 8 consisting of 100 mM HEPES (pH7.6), 100 mM NaCl, 0.2 mM EDTA, 0.05% NaN3

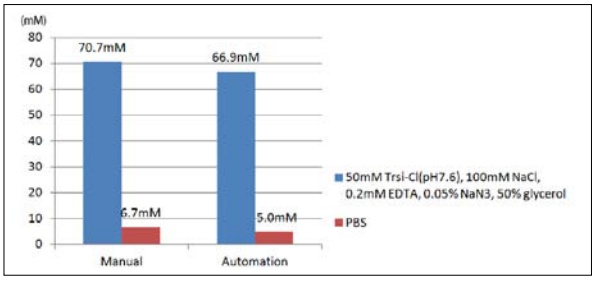


Figure 2. Comparison of efficiency between manual and automated dialysis.
Blue and darkbrown bar show the dialysis efficiency of buffer consisting of 50 mM Tris-Cl (pH7.6), 100 mM NaCl, 0.2 mM EDTA, 0.05% NaN3, and PBS buffer, respectively.

Ordering Information

Cat. no.	Product Description
K-7240	*ExiProgen*™ Dialysis Kit, 16 rxns
Related Product	
D-2020	*AccuLadder*™ Protein Size Marker (Low), 500 µl
D-2010	*AccuLadder*™ Protein Size Marker (Broad), 500 µl
A-5041	*ExiProgen*™, Instrument

02

Manual Protein Expression and Purification: *AccuRapid*™ & *MagListo*™

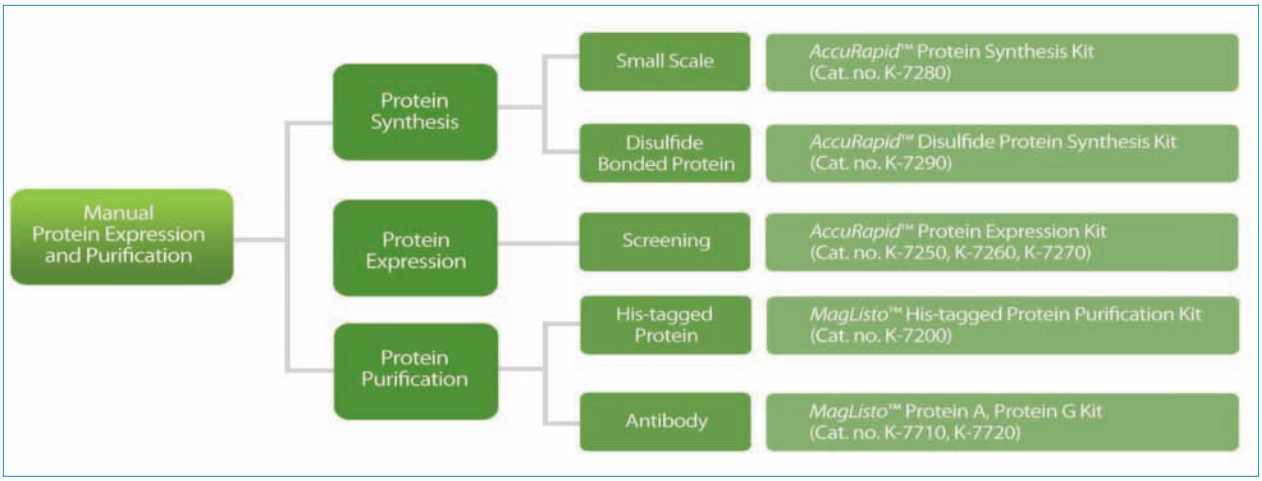
Overview

These manual product groups are divided into three categories.

1) Protein Synthesis (expression and purification) - *AccuRapid*™ Protein Synthesis Kit and *AccuRapid*™ Disulfide Protein Synthesis Kit

2) Protein Expression only - *AccuRapid*™ Cell-Free/Midi/Maxi Protein Expression Kit

3) Protein Purification only - *MagListo*™ His-tagged Protein Purification Kit, *MagListo*™ Protein A, Protein G Kit



AccuRapid™ Protein Synthesis Kit

The Easy Method for Protein Synthesis!

Description

AccuRapid™ Protein Synthesis Kit uses a manual procedure for protein expression and purification. It includes all components required for cell-free protein expression using *E. coli* extract and Ni-NTA affinity purification. Target protein is recovered in up to 100 µg per reaction.

Features and Benefits

- Simplicity with High Performance
  You can synthesize protein easily in a tube without using living cell expression system and also can obtain proteins that are hard to get through a conventional cell expression method.
- Flexibility
  Any template DNA containing T7 promoter and terminator can be used.
- Compatibility with automation
  This kit is compatible with *ExiProgen*™ EC Protein Synthesis Kit.

Application

This kit can be used in protein research and development including enzyme for research and business.

Experimental Datata

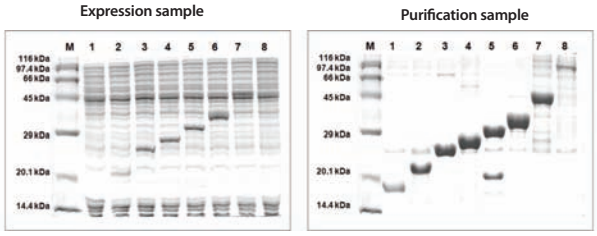


Figure 1. SDS-PAGE data of various proteins synthesized from various templates.

M: *AccuLadder*™ Protein Size Marker (Broad)

1: CalmL3 (17.5 kDa) 2: DUSP 3 (22 kDa) 3: CAT (24 kDa)
4: AcGFP (29 kDa), 5: ERFP (31 kDa) 6: EF-Ts (34 kDa)
7: VF (45 kDa) 8: BM3 (117 kDa)

Ordering Information

Cat. no.	Product Description
K-7280	*AccuRapid*™ Protein Synthesis Kit, 5 rxns
Related Product	
K-3030	*AccuPrep*® Plasmid Extraction Kit, 200 rxns
K-3034	*AccuPrep*® PCR Purification Kit, 200 rxns
K-3035	*AccuPrep*® Gel Purification Kit, 200 rxns
D-1030	100 bp DNA Ladder, 250 µl (135 ng/µl)
D-1040	1 kb DNA Ladder, 500 µl (130 ng/µl)
D-2020	*AccuLadder*™ Protein Size Marker (Low), 500 µl
D-2010	*AccuLadder*™ Protein Size Marker (Broad), 500 µl
K-7350	pBIVT Vectors Set-1
K-7400	*ExiProgen*™ ProXpress PCR Template Kit, 16 rxns

Screening for Protein Expression

Description

AccuRapid™ Protein Expression Kit Kit uses a manual procedure for protein expression. This kit contains an optimized *E. coli* extract containing T7 RNA polymerase for transcription and all necessary components for translation. When combined with the master mix supplied in the kit, all other required components including amino acids, rNTPs, and appropriate salts are provided enabling the expression of high levels of recombinant proteins. This flexible system expresses up to 300 µg/ml of protein within only 3 hours from the template DNA which contains T7 promoter, T7 terminator and RBS (ribosomal binding site).

Features and Benefits

- High Speed
  Protein can be expressed within just 3 hours from template DNA.
- Flexibility
  Any template DNA containing T7 promoter/terminator and RBS can be used.
- Expression of cytotoxic protein
  This kit expands its application even to proteins that have cytotoxic effects on cells. Those proteins are hard to be produced through cell-based protein expression system.
- Compatibility
  This kit is compatible with most *ExiProgen*™ EC related Kits and used to determine for the optimum concentration of DNA.

Application

This kit can be used in protein research and development including enzyme for research and business.

Experimental Data

1. AcGFP (Positive Control) expression using *AccuRapid*™ Protein Expression Kits

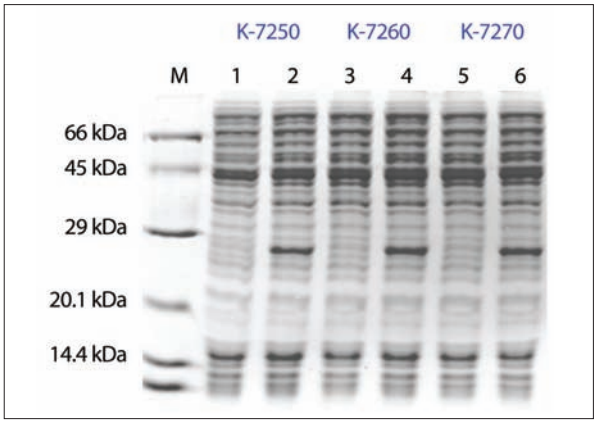


Figure 1. Positive control protein expression data of *AccuRapid*™ Protein Expression Kits. (SDS-PAGE and Coomassie Brilliant Blue staining)

M: *AccuLadder*™ Protein Size Marker (Low) (Bioneer Cat.no. D-2020)
1,3,5: Negative control(No-DNA)
2,4,6: Positive control (pBIVT-AcGFP)
Note) K-7250 - 45 µl rxn, K-7260 - 1 ml rxn, K-7270-10 ml rxn
All samples are loaded 1.25 µl from final reactant.

2. Expression from various protein

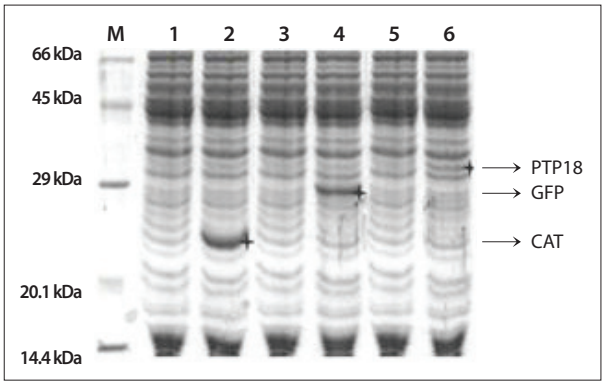


Figure 2. Various protein expression data (SDS-PAGE and Coomassie Brilliant Blue staining)

M: *AccuLadder*™ Protein Size Marker (Low) (Bioneer Cat. no. D-2020)
1,3,5: Negative control (No-DNA)
2: CAT (Chloramphenicol acetyl transferase)
4: GFP (Green fluorescence protein)
6: PTP1B(Protein tyrosine phosphatase 1B)

3. Template DNA의 최적 DNA 농도 screening

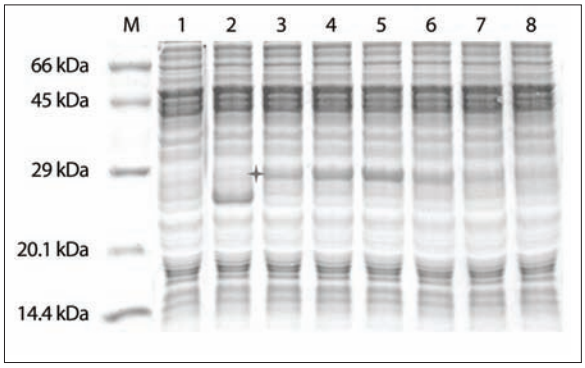


Figure 3. Determination of the optimal amount of template DNA for the expression of pBIVT-GFP.
(SDS-PAGE and Coomassie Brilliant Blue staining)
M: *AccuLadder*™ Protein Size Marker (Low) (Bioneer Cat. no. D-2020)
1: Negative Control(No-DNA)
2: CAT (Chloramphenicol acetyl transferase)
3 ~8: pBIVT-GFP(50, 100, 200, 400, 600, 800 ng)

Ordering Information

Cat. no.	Product Description
K-7250	*AccuRapid*™ Cell-Free Protein Expression Kit, 45 µl x 24 rxns
K-7260	*AccuRapid*™ Midi Protein Expression Kit, 1 ml x 5 rxns
K-7270	*AccuRapid*™ Maxi Protein Expression Kit, 10 ml x 1 rxn
Related Product	
K-3030	*AccuPrep*® Plasmid Extraction Kit, 200 rxns
K-3034	*AccuPrep*® PCR Purification Kit, 200 rxns
K-3035	*AccuPrep*® Gel Purification Kit, 200 rxns
D-1030	100 bp DNA Ladder, 250 µl (135 ng/µl)
D-1040	1 kb DNA Ladder, 500 µl (130 ng/µl)
D-2020	*AccuLadder*™ Protein Size Marker (Low), 500 µl
D-2010	*AccuLadder*™ Protein Size Marker (Broad), 500 µl
K-7350	pBIVT Vectors Set-1
K-7400	*ExiProgen*™ ProXpress PCR Template Kit, 16 rxns

AccuRapid™ Disulfide Protein Synthesis Kit[NEW]

The Easy Method for Disulfide-bonded Protein Synthesis!

Description

AccuRapid™ Disulfide Protein Synthesis Kit is used for synthesis of proteins containing disulfide bonds. This kit includes all components required for cell-free protein expression using *E. coli* extract and Ni-NTA affinity purification.

Features and Benefits

- Protein Synthesis with Disulfide-boned
  This kit is used to synthesize a protein that has two or more disulfide bonds, providing its intact function.
- Flexibility
  Any template DNA containing T7 promoter and terminator can be used.
- Compatibility
  This kit is compatible with *ExiProgen*™ EC-Disulfide Protein Synthesis Kit.

Application

This kit is can be used to produce pharmaceutical protein and scFv for antibody screening.

Experimental Data

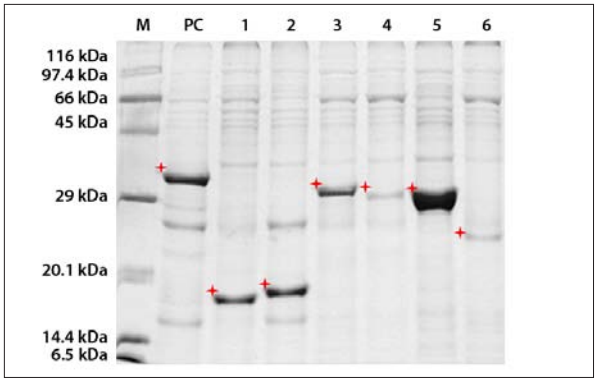


Figure 1. Expression and purification of antibody fragment with *AccuRapid*™ Disulfide Protein Synthesis Kit. (12% SDS PAGE gel stained with Coomassie Blue R-250 and samples were loaded by 7.5% in one well).

M: *AccuLadder*™ Protein Size Marker (Broad, Bioneer, D-2010)

PC: positive control DNA (single-chain variable fragment)

1: VL (Variable domain of light chain)

2: VH (Variable domain of heavy chain)

3: LC (Light chain)

4: VH-CH1 (Variable & conserved domain 1 of heavy chain)

5: VL- Lκ(Variable domain of light domain & light kappa chain)

6: VH-Lκ(Variable domain of Heavy chain & light kappa chain)

Ordering Information

Cat. no.	Product Description
K-7290	*AccuRapid*™ Disulfide Protein Expression Kit, 5 rxns
Related Product	
K-7330	*ExiProgen*™ EC-Disulfide Protein Synthesis Kit, 8rxn
K-3030	*AccuPrep*® Plasmid Extraction Kit, 200 rxns
D-1030	100 bp DNA Ladder, 250 µl (135 ng/µl)
D-1040	1 kb DNA Ladder, 500 µl (130 ng/µl)
D-2020	*AccuLadder*™ Protein Size Marker (Low), 500 µl
D-2010	*AccuLadder*™ Protein Size Marker (Broad), 500 µl
K-7350	pBIVT Vectors Set-1

02 *MagListo*™ His-tagged Protein Purification Kit

Fast Protein Purification or Screening of His-tagged Protein

Description

MagListo™ His-tagged protein Purification Kit consists of Ni-NTA Magnetic Beads and buffer for His-tagged protein purification. Ni-NTA Magnetic Beads are silica-coated magnetic particles with a functional group of Ni^{2+}-nitrilotriacetic acid (NTA), which possesses an affinity to a histidine tagged protein. The Nanobeads' 400 nm diameter in average provides a large surface area which gives high binding capacity, and their strong magnetic forces allow for short hands-on time as well as increase the yield of target molecules.

Features and Benefits

- High Speed
  The entire purification is complete in about 30 minutes.
- High Performance
  Highly pure proteins are obtained through our exclusive SSMB (Spherical-Shape Magnetic Beads).
- High Efficiency
  High Efficiency is shown in screening process from crude cell lysate.

Specification

	Mini scale	Midi scale	Maxi scale
Processing time	~5 min	~17 min	~26 min
Typical elution volume	0.2 ml	2 ml	5 ml
Binding capacity	30~40 mg his-tagged protein/1 g Ni-NTA beads		

Application

This kit is manual type and is can be used to purify target protein with His-tag.

Experimental Data

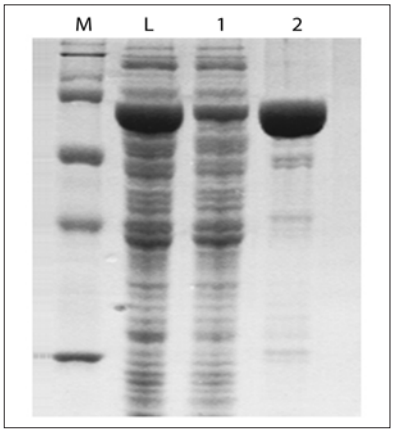


Figure 1. Purification of 6x His-tagged protein.
M: Protein broad marker (Bioneer, D-2010)
1: Loading sample
2: Unbound sample
3: Eluted sample (*MagListo*™ His-tagged Protein Purification Kit)

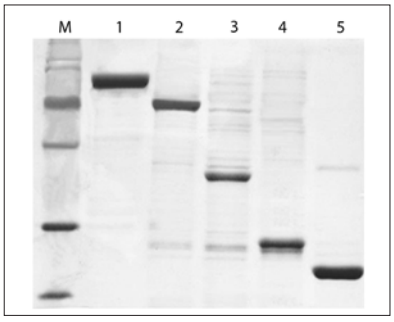


Figure 2. Purification of 5 his-tagged proteins with various molecular weight.
M : Protein broad marker (Bioneer, D-2010)
1: Size 94 kDa His-tagged protein
2: Size 67 kDa His-tagged protein
3: Size 38 kDa His-tagged protein
4: Size 27 kDa His-tagged protein
5: Size 25 kDa His-tagged protein

Ordering Information

Cat. no.	Product Description
K-7200	*MagListo*™ His-tagged Protein Purification Kit,1 ml x 5(10%)
Related Product	
TA-1017-1	*AccuNanoBead*™ Ni-NTA Magnetic Beads, size 400 nm, 0.5 g/25 ml
D-2020	*AccuLadder*™ Protein Size Marker (Low), 500 µl
D-2010	*AccuLadder*™ Protein Size Marker (Broad), 500 µl

MagListo™ Protein A, Protein G Kit

Fast and Easy Purification of Antibody

Description

MagListo™ Protein G Kit and *MagListo*™ Protein A Kit consist of Protein G or Protein A Magnetic Nanobeads, respectively, with buffers for antibody purification. Magnetic Nanobeads of each product are silica-coated magnetic particles further conjugated with highly pure Protein A or Protein G. The Nanobeads' 400 nm average diameter provides a large surface area which gives high binding capacity, and their strong magnetic forces allow for short hands-on time as well as increase the yield of target molecules.

Features and Benefits

- Fast Binding
  Powerful magnetism reduces experiment time and increases yield.
- Large Surface Area
  Average diameter of 400 nm provides large surface and allows high binding capacity.
- Specificity
  Globular beads reduce non-specific binding.

Application

Antibody purification, Antigen purification, Immunoprecipitation, Protein-protein interaction, and Cell separation etc.

Experimental Data

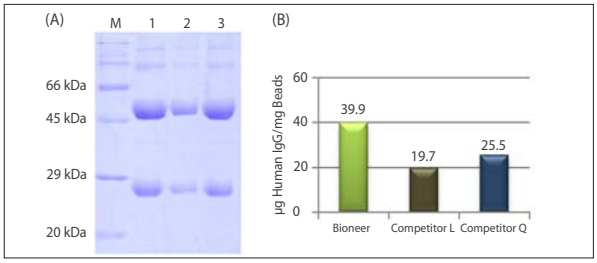


Figure 1. Comparison of protein binding efficiency of *MagListo*™ protein G Kit with competitor's kits.

A) Upper and lower bands are heavy (HC) and light chain (LC) of purified human IgG in SDS-PAGE gel, respectively.
M: Protein size marker (low), 1: Bioneer, 2: Competitor L, 3: Competitor Q

B) Amount of bound IgG is quantified by UV-spectrophotometer. All reactions are carried out for 1 hr at room temperature. Human IgG is purchased from Sigma (Cat. no. I4506).

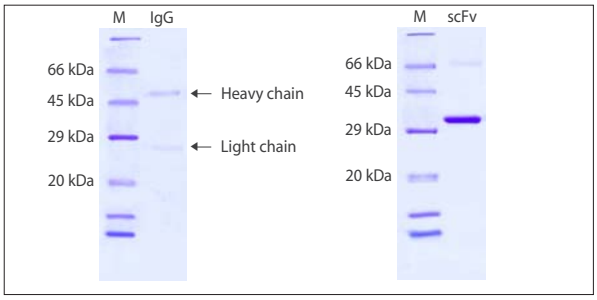


Figure 2. Purification of IgG and scFv using *MagListo*™ Protein A Kit

Specification

	Protein G Magnetic Nanobeads	Protein A Magnetic Nanobeads
Matrix	Silica-coated Fe_3O_4	Silica-coated Fe_3O_4
Concentration	40 mg/ml (4% suspension)	40 mg/ml (4% suspension)
Binding capacity	> 30 μg Human IgG/mg beads	> 20 μg Human IgG/mg beads

Ordering Information

Cat. no.	Product Description
K-7710	*MagListo*™ Protein G Kit, 1 x 1 ml
K-7720	*MagListo*™ Protein A Kit, 1 x 1 ml
Related Product	
TA-1021-1~3	*AccuNanoBead*™ Protein G Magnetic Nanobeads, size 400 nm, 40 mg/1 ml, 200 mg/10 ml, 500 mg/25 ml
TA-1022-1~3	*AccuNanoBead*™ Protein A Magnetic Nanobeads, size 400 nm, 40 mg/1 ml, 200 mg/10 ml, 500 mg/25 ml

03

Preparation of Template DNA for Protein Expression

Overview

Template DNA is prepared to express target protein of interest using *ExiProgen*™. Template DNA includes T7 promoter, ribosome binding site (RBS), target gene insert with 6X his-tag sequence, and followed by T7 terminator sequence. The target gene has to include start codon and stop codon. Presence of 6X his-tag sequence is essential for purification of the target protein selectively using the Ni-NTA magnetic bead. Within the insert, the 6X his-tag can be positioned adjacent to the start codon in the N terminus or the stop codon in the C terminus.

For vector selection, one recommendation is to use the in-vitro pBIVT vector (Cat. no. K-7350) from Bioneer. Other set of vectors such as pK7, pIVEX, pET, etc can also be used.

Using *ExiProgen*™ ProXpress PCR Template Kit (Cat. no. K-7400, 7401), you can prepare template DNA from PCR product without cloning process. This kit is very useful for screening of various proteins with small amount in short time. With the kit, it takes one day to prepare template DNA from genomic DNA, cDNA, or T-vector and synthesize protein. Similarly *ExiProgen*™ Circular PCR Template Kit (Cat. No. K-7410 & K-7411) increases efficiency of target protein expression from template DNA generated by PCR reaction.

Bioneer also provides its customer Gene Synthesis Service which supplies a vector including target DNA for in vitro translation. Please refer to detailed contents at our homepage (http://eng.bioneer.com/products/GeneSynthesis/GeneSynthesisService-overview.aspx).

1) NH constructs (5'end 6x His-tagged template)

2) CH constructs (3'end 6x His-tagged template)

ExiProgen™ ProXpress PCR Template Kit

PCR-mediated quick preparation of template DNA for protein expression

Description

This kit is used to synthesize a linear template DNA by PCR without cloning process. The kit contains all reagent required for synthesis of template DNA, such as second PCR primer set, N/C terminus cassette, and PCR premix, except a first PCR primer set to amplify a target gene. The cassettes include T7 promoter, ribosome binding site, and T7 terminator sequence. The synthesized template DNA for protein expression can be used with Bioneer's in vitro protein synthesis products.

Features and Benefits

- Rapid & Easy Preparation of Linear Template DNA (for Protein Expression)
  Template DNA is easily synthesized by just two step of PCR from cDNA or genomic DNA in about 7 hours. Therefore, target protein can be produced within a day using this kit with *ExiProgen*™.
- Accurate Protein Template by High Fidelity PCR
  Because this kit uses *AccuPower*™ *ProFi Taq* PCR PreMix as long & high fidelity PCR premix, you can obtain a high quality template DNA with a minimal occurrence of error in the sequence.
- High Efficiency
  This kit has been thoroughly optimized to *ExiProgen*™; therefore produced template of DNA can produce large amount of protein (up to 100 μg per 1 rxn in *ExiProgen*™).

Application

Preparation of template DNA for in vitro protein synthesis in *ExiProgen*™.

Experimental Data

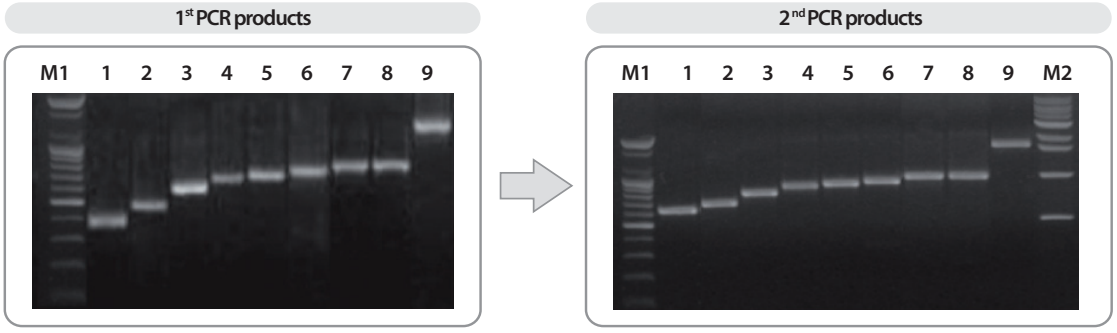


Figure 1. Agarose gel data of template DNA synthesized by each PCR.
Synthesis of linear template DNA with *ExiProgen*™ ProXpress PCR Template Kit.
M1: 100 bp DNA Ladder (D-1030, Bioneer), M2: 1 kb DNA Ladder (D-1040, Bioneer)
1: SAV (Template - pT), 2: RNase H (Template - BL21 (DE3) gDNA), 3: hGH (Template - pT)
4: CAT (Template - pBIVT), 5: UDG (Template - BL21 (DE3) gDNA), 6: AcGFP (Template - pBIVT)
7: EVO (Template - pT), 8: RFP (Template - pIVEX), 9: Poly A polymerase (Template - pET15b)

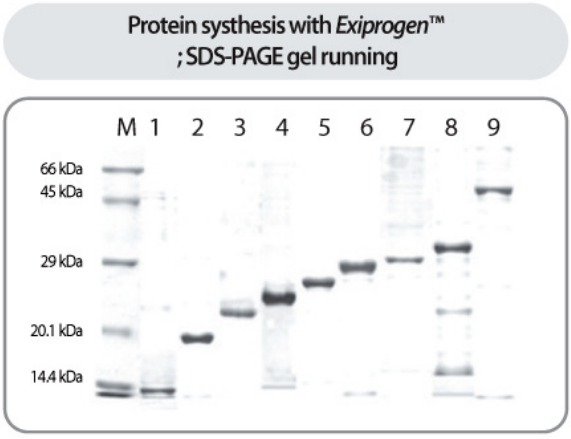


Figure 2. SDS-PAGE gel data of synthesized proteins.

Each linear template DNA generated by *ExiProgen*™ ProXpress PCR Template Kit and those are used as template for protein synthesis with *ExiProgen*™ EC Protein Synthesis Kit.

M: *AccuLadder*™ Protein Size Marker (Low) (D-2020, Bioneer)

1: SAV (13 kDa), 2: RNase H (20 kDa), 3: hGH (23 kDa), 4: CAT (26.5 kDa), 5: UDG (28 kDa)

6: AcGFP (28 kDa), 7: EVO (30 kDa), 8: RFP (31 kDa), 9: Poly A polymerase (54 kDa)

Ordering Information

Cat. no.	Product Description
K-7400	*ExiProgen*™ ProXpress PCR Template Kit, 16 rxns
K-7401	*ExiProgen*™ ProXpress PCR Template Kit, 32 rxns
Related Product	
N-8229	1^{1st} primer F/R sets (N-terminus 6x His-tag), each 5 nmole
N-8230	1^{1st} primer F/R sets (C-terminus 6x His-tag), each 5 nmole
K-2631	*AccuPower® ProFi Taq* PCR PreMix, 96 tubes, 20 µl rxn/tube
K-3034	*AccuPrep®* PCR Purification Kit, 200 rxns
K-3035	*AccuPrep®* Gel Purification Kit, 200 rxns
D-1030	*AccuLadder*™ 100 bp DNA Ladder, 250 µl (135 ng/µl)
D-1040	1 kb DNA Ladder, 500 µl (130 ng/µl)

ExiProgen™ Circular PCR Template Kit[NEW]

Degradation-resistant template DNA preparation for protein expression

Description

This kit is used to synthesize a circular template DNA for cell-free protein expression by PCR without cloning process. The kit contains all reagent required for synthesis of template DNA, such as second PCR primer set, N/C terminus cassette, and PCR premix, except a first PCR primer set to amplify a target gene. The cassettes include T7 promoter, ribosome binding site, and T7 terminator sequence. The synthesized circular template DNA is so stable that target protein is expressed effectively without degradation of template DNA, even if it takes long time. The synthesized template DNA for protein expression can be used with Bioneer's in vitro protein synthesis products.

Features and Benefits

- Rapid & Easy Preparation of Circular Template DNA (for Protein Expression)
  A circular template DNA is easily synthesized by three step of PCR from cDNA or genomic DNA. Therefore, target protein can be produced within a day using this kit with *ExiProgen*™.
- Enhanced Stability
  A linear PCR product can be degraded by nucleases during cell-free protein expression, which decrease yield of target protein. This kit resolves the problem of template degradation and enhance the efficiency of target protein expression.
- Accurate Protein Template by High Fidelity PCR
  AccuPower™ *ProFi Taq* PCR PreMix, which has long & high fidelity PCR nature, confers high accuracy in products of template DNA.
- High Efficiency
  This kit has been thoroughly optimized to *ExiProgen*™; therefore produced template of DNA can produce large amount of protein (up to 100 µg per 1 rxn in *ExiProgen*™).

Application

Preparation of template DNA with high stability for long-time in vitro protein synthesis in *ExiProgen*™.

Recommendation for using *ExiProgen*™ Kit

Template DNA Kit	Available Protein Synthesis Kit
ExiProgen™ ProXpress PCR Template Kit	- *ExiProgen*™ EC Protein Synthesis Kit
ExiProgen™ Circular PCR Template Kit	- *ExiProgen*™ EC Protein Synthesis Kit - *ExiProgen*™ EC-Maxi Protein Synthesis Kit - *ExiProgen*™ EC-Tagfree Protein Synthesis Kit - *ExiProgen*™ EC-Disulfide Protein Synthesis Kit

Experimental Data

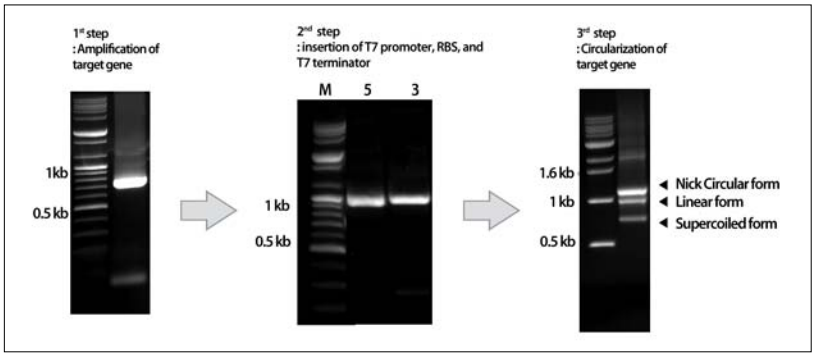
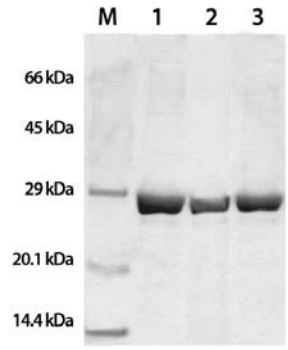


Figure 1. Agarose gel data of template circular DNA synthesized by three steps.
Cirular PCR template is synthesized with *ExiProgen*™ Cirular PCR Template Kit.



AcGFP	(mg/mL)	Total (mL)	Total (μg)	
Plasmid DNA	2.290	0.21	481	100.0%
Linear DNA	0.564	0.21	118.4	24.6%
Circular DNA	1.646	0.21	345.8	71.8%

Figure 2. The performance test of *ExiProgen*™ Circular PCR Template Kit using *ExiProgen*™ EC-Maxi Protein Synthesis Kit.

M: protein marker
1: protein synthesis from 6 μg of plasmid DNA
2: protein synthesis from 0.3 μg of linear DNA
3: protein synthesis from 0.3 μg of circular DNA

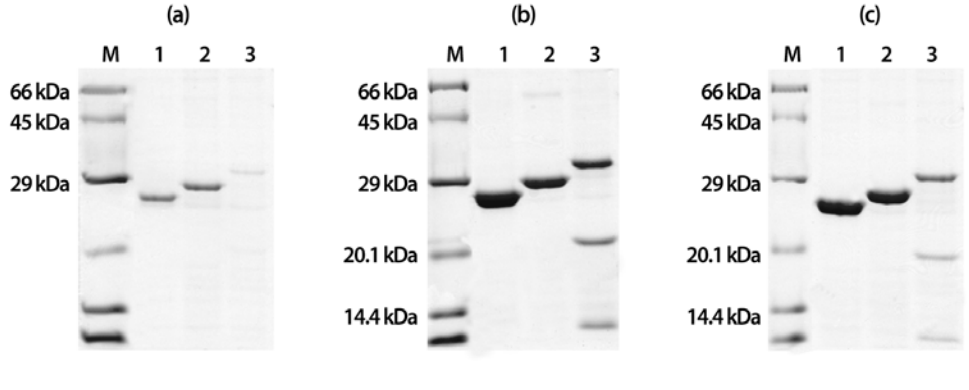


Figure 3. The performance test of *ExiProgen*™ Circular PCR Template Kit with various proteins using *ExiProgen*™ EC-Maxi Protein Synthesis Kit.

(a) Linear DNA, (b) Circular DNA, (C) Plasmid DNA

M: protein marker, 1: CAT (23 kDa), 2: AcGFP (29 kDa), 3: RFP (31 kDa)

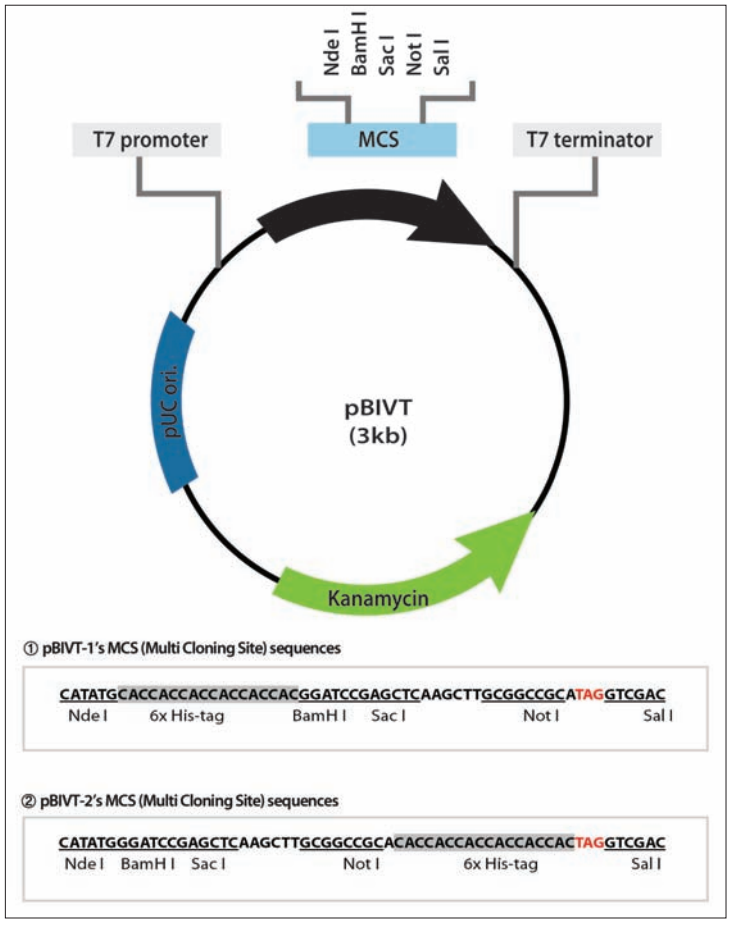
Ordering Information

Cat. no.	Product Description
K-7410	*ExiProgen*™ Circular PCR template Kit , 16 rxns
K-7411	*ExiProgen*™ Circular PCR template Kit , 32 rxns
Related Product	
N-8229	1[1st] primer F/R sets (N-terminus 6x His-tag), each 5 nmole
N-8230	1[1st] primer F/R sets (C-terminus 6x His-tag), each 5 nmole
K-2631	*AccuPower® ProFi Taq* PCR PreMix, 96 tubes, 20 μl rxn/tube
K-3034	*AccuPrep®* PCR Purification Kit, 200 rxns
K-3035	*AccuPrep®* Gel Purification Kit, 200 rxns
D-1030	*AccuLadder*™ 100 bp DNA Ladder, 250 μl (135 ng/ μl)
D-1040	1 kb DNA Ladder, 500 μl (130 ng/μl)

Vector for Bioneer Cell-free protein expression for vector

Description

pBIVT Vector Set-1 is compatible with all cell-free protein expression with T7 protein expression system, and synthesis product family of kits, your gene of interest can be fused to his-tag at its 5′- (pBIVT-1) or 3′- (pBIVT-2) end using each of the set. Schematic structure of the vector is shown below.



① pBIVT-1's MCS (Multi Cloning Site) sequences

CATATGCACCACCACCACCACCACGGATCCGAGCTCAAGCTTGCGGCCGCATAGGTCGAC
Nde I 6x His-tag BamH I Sac I Not I Sal I

② pBIVT-2's MCS (Multi Cloning Site) sequences

CATATGGGATCCGAGCTCAAGCTTGCGGCCGCACACCACCACCACCACCACTAGGTCGAC
Nde I BamH I Sac I Not I 6x His-tag Sal I

Application

DNA vector construction as template for cell-free protein synthesis with T7 system.

Ordering Information

Cat. no.	Product Description
K-7350	pBIVT Vector Set-1
Related Product	
K-3030	*AccuPrep*® Plasmid Extraction Kit, 200 rxns
K-3034	*AccuPrep*® PCR Purification Kit, 200 rxns
D-1030	*AccuLadder*™ 100 bp DNA Ladder, 250 µl (135 ng/µl)
D-1040	1 kb DNA Ladder, 500 µl (130 ng/µl)
E-3061	T4 DNA Ligase, 20,000 U

04

Protein Service

Protein Synthesis Service

Gene to Protein Service

04 Standard Protein Synthesis Service

Description

Bioneer's Standard Protein Synthesis Service provides recombinant protein in as little as 1 week. The service is using the automatic cell-free protein expression and Ni-NTA affinity protein purification system (*ExiProgen*™). And the service enables synthesis of various proteins such as enzyme, growth factor, hormone, antigen protein and antibody fragments.

Features and Benefits

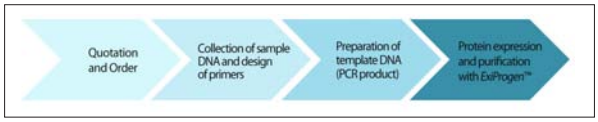
- Fast Process
  You can receive target proteins in as little as 1 week.
- High Performance
  This service enables synthesis of various proteins enzyme, growth factor, hormone, antigen protein, antibody fragments, and toxic protein to *in vivo* expression systems.
- Compatibility with *ExiProgen*™
  ExiProgen™ is used for this service. Therefore customers can generate their own protein by themselves through the instrument.

Service Procedure

Type I) *E. coli* expression vectors (ex. T7-based expression vector)

Note) Available for gene of 0.3-3 kb in ORF (open reading frame)

Type II) Non-expression vectors (ex. Cloning vector/target gene)



Note) Available for gene of 0.3-2 kb in ORF (open reading frame)

Ordering Information

Amount of sample	*E. coli* expression vector[1]	10 µg ($A_{260/280}$: 1.7-2.0, $A_{260/230}$: >1.5)
	Non-expression vector[2]	2 µg
Cost of synthesis	$600	
	Note) In the case of no final product occurred, only $100 set-up charge will be billed.	
Delivery form[3]	1. Data report - E-mail 2. Protein - 100 µg (0.1-1.0 mg/ml of solution form)	
Period of synthesis[4]	7 - 14 working days	
Increase in quantity[5]	$100 - 300/additional 100 µg of protein	

1) If amount and purity of plasmid DNA are inconsistent with sample condition, an increase in quantity service of DNA will proceed and additional cost will be incurred.
2) Non-expression vector means samples such as cloning vector or PCR product. These samples require process of template DNA preparation for protein expression and therefore additional cost ($100) will be charged.
3) The 100 µg means amount of total protein in solution.
4) The period may increase according to status of DNA sample or amount of protein and shipping period of sample is excluded from the period.
5) The cost is commensurate with levels of difficulty.

04 One-day Protein Synthesis Service

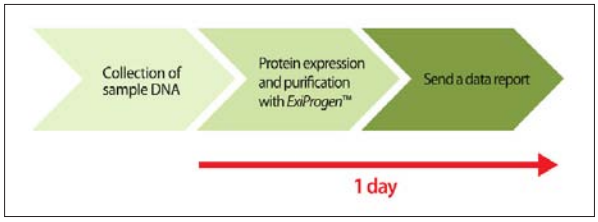
Description

The service is the special service using the automatic cell-free protein synthesis system (*ExiProgen*™ & *ExiProgen*™ EC Protein Synthesis Kit) and enables synthesis of various proteins such as enzyme, growth factor, hormone, antigen protein and antibody.

Features and Benefits

- Fast Process
  You can receive a data report to protein synthesis in a day.
- High Performance
  This service enables synthesis of various proteins enzyme, growth factor, hormone, antigen protein, and toxic to *in vivo* expression systems.
- Compatibility
  If necessary, you can synthesize target protein yourself because this service is using Bioneer's *ExiProgen*™ EC Protein Synthesis Kit.

Service Procedure



Ordering Information

Amount of sample[1)]	*E. coli* expression vector[1)]	10 µg ($A_{260/280}$: 1.7-2.0, $A_{260/230}$: >1.5)
Cost of synthesis	$300	
	Note) If protein synthesis fails, you will pay only set-up charge ($100).	
Delivery form[2)]	1. Data report - E-mail 2. Protein - 0.1-1.0 mg/ml of solution	
Period of synthesis[3)]	1 day	

1) If amount and purity of plasmid DNA are inconsistent with sample condition, an increase in quantity service of DNA will proceed and additional cost will be incurred.

2) We will provide synthesized protein from once a reaction. If you want more protein, the service can be converted into "Standard Protein Synthesis Service".

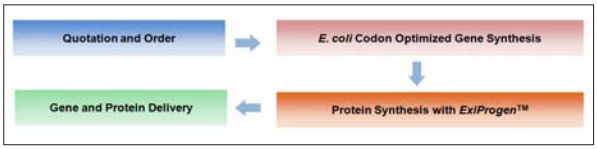
3) Shipping period of sample to a customer is excluded from the period.

04 Standard Gene to Protein Service

Description

This service is based on Bioneer's Gene Synthesis Service (Please refer to E part in this catalog). Your protein of target can be synthesized fully by Bioneer's *ExiProgen*™ through its gene synthesis followed by protein synthesis. Synthesized gene will be cloned to E. coli expression vector and then it will be connected to Protein Synthesis Service.

Schematics of service process



Features and Benefits

- High Performance
  This service enables synthesis of various proteins enzyme, growth factor, hormone, antigen protein, antibody, and toxic to in vivo expression systems.
- High Quality
  Every synthetic gene is 100% confirmed by sequencing.
- Codon Optimization Service
  Synthesized target gene is optimized for *E. coli* codon system to enhance protein expression and function.
- Compatibility
  If necessary, you can synthesize target protein yourself because this service is using Bioneer's protein synthesis kits.

Ordering Information

Cost of synthesis[1]		$1,200 (Normal sequence/300 bp-1 kb)	
Additional cost of gene synthesis[2]		Gene synthesis - $0.45/bp (>1 kb)	
Period of synthesis		1-1,500 bp	20-35 working days
		1,501-3,000 bp	30-40 working days
Delivery form	Gene	Plasmid DNA (lysophilized form) ; 2-5 μg	
	Protein[3]	100 μg (0.1-1.0 mg/ml) of solution	

1) You will be charged 50% of your quotation price, if you cancel your order within 5 days after you receive confirmation of order e-mail from Bioneer, After 5 days of confirmation e-mail, 80% of your quotation price will be charged.

2) Additional cost will apply for gene segments containing complexities such as high or low GC content, repeat sequences or homopolymeric runs.

3) If protein synthesis fails, you will pay only set-up charge ($100). But, synthesized gene will deliver to you and cost of gene synthesis will be charged.

04 Cloning-free Gene to Protein Service

Description

This service is based on Bioneer's AccuGeneBlock Service. We generate PCR product first, which can be used for protein synthesis. Therefore, this service is faster than Standard Gene to Protein Service.

Features and Benefits

- Fast Process
  You can receive genes and proteins in as little as 2 weeks. Because this service is not necessary cloning and cell culture process, PCR product will be used to protein synthesis directly.

- Codon Optimization Service
  Synthesized target gene is optimized for *E. coli* codon system to enhance protein expression and function.

- Compatibility
  If necessary, you can synthesize target protein yourself because this service is using Bioneer's protein synthesis kits.

Saving time with Bioneer's Gene to Protein service

Ordering Information

Cost of synthesis		$1,000 (Normal sequences/300 bp-1 kb)
Period of synthesis		10-15 working days
Delivery form	Gene	PCR product (lyophilized form) ; 500 ng-1μg
	Protein[1)]	100 μg (0.1-1.0 mg/ml) of solution

1) If protein synthesis fails, you will pay only set-up charge ($100). But, synthesized gene will deliver to you and cost of gene synthesis will be charged.