ExiProgen™ Protein Expression Optimization Kit

(V1/2021-07-12)

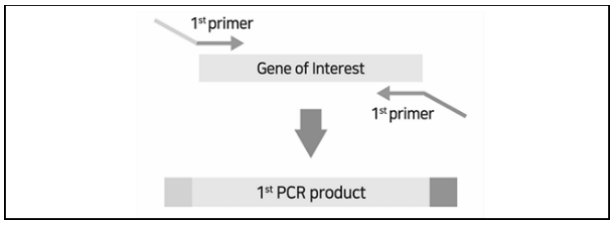
[Cat. No.] K-7410

Introduction

ExiProgen™ Protein Expression Optimization Kit rapidly generates multiple linear template DNAs using a two-step PCR process. The template DNAs include essential elements for protein expression and purification, along with sequences for 6 types of tags to optimize the protein expression conditions. The kit enables the production of 6 templates (1 control DNA with tag 1 to 6) of more than 1 μg and 30 templates (5 target genes with tag 1 to 6).

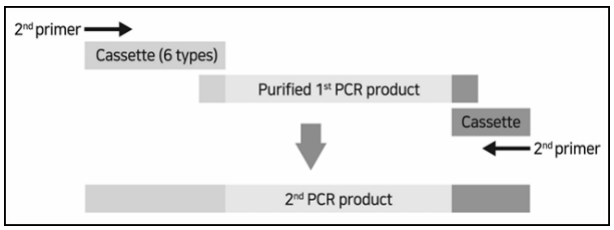
A. First PCR

In the first PCR, target genes are amplified and partial sequences of cassettes are added.



B. Second Overlapping PCR

In the second PCR, the cassettes are added to the upstream and downstream of first PCR products. The cassettes are DNA fragments containing sequences of the T7 promoter, ribosomal binding site, six histidine tag, one of the 6 types of tag, TEV cleavage site and T7 terminator.



Features & Benefits

- Convenient: Contains all PCR components for generating template DNA for screening optimal expression conditions.
- Rapid: Saves time by getting the template DNA through PCR instead of time-consuming cloning steps.
- Minimized PCR error: Provides *AccuPower*® *ProFi Taq* PCR PreMix, having high accuracy and precision, to lower the error rate as much as possible.

Components

Components	Concentration	Amount
AccuPower® *ProFi Taq* PCR Premix	-	50 μl x 96 tubes
Tag 1_Cassette mixture	7.5 ng/μl	70 μl x 1 ea
Tag 2_Cassette mixture	7.5 ng/μl	70 μl x 1 ea
Tag 3_Cassette mixture	7.5 ng/μl	70 μl x 1 ea
Tag 4_Cassette mixture	7.5 ng/μl	70 μl x 1 ea
Tag 5_Cassette mixture	7.5 ng/μl	70 μl x 1 ea
Tag 6_Cassette mixture	7.5 ng/μl	70 μl x 1 ea
Control DNA	10 ng/μl	10 μl x 1 ea
1st Forward primer (for Control DNA)	10 pmol/μl	10 μl x 1 ea
1st Reverse primer (for Control DNA)	10 pmol/μl	10 μl x 1 ea
2nd Forward primer	10 pmol/μl	220 μl x 1 ea
2nd Reverse primer	10 pmol/μl	220 μl x 1 ea

*** Note:** Refer to experimental procedures to prepare a set of gene-specific primers to amplify the target gene before using the kit.

Specifications

Expression enhanced tags			
Tag 1	-	Tag 4	Ubiquitin
Tag 2	Expressivity	Tag 5	Trx
Tag 3	S	Tag 6	SNUT

Storage

Store at a temperature between -70°C and -20°C.

Online Resources

Korean **English**

Visit our **product page** for additional information and protocols

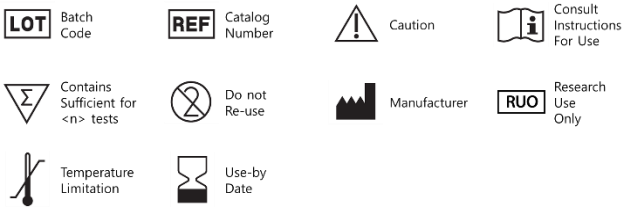
Ordering Information

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

Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Explanation of Symbols



Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td>1</td><td> 
Primer design</td><td>
1. Prepare the gene-specific primers as shown below.
<table>
<tr><th>Primers</th><th>Sequences (5' to 3')</th></tr>
<tr><td>1st Forward primer (48-mer)</td><td>GAGCTCGAAAACTTATATTTTCAGGGC + 21-mer from the target gene's 5' end</td></tr>
<tr><td>1st Reverse primer (48-mer)</td><td>GGGCTTTGTTAGCAGCCGGTCGACCTA + 21-mer from the target gene's 3' end in reverse complementary sequence</td></tr>
</table>
• Primers for control DNA
<table>
<tr><th>Primers</th><th>Sequences (5' to 3')</th></tr>
<tr><td>1st Forward primer (for Control DNA)</td><td>GAGCTCGAAAACTTATATTTTCAGGGC + ATGGAAATTAAATGTGTTAAT (21-mer)</td></tr>
<tr><td>1st Reverse primer (for Control DNA)</td><td>GGGCTTTGTTAGCAGCCGGTCGACCTA + CTCGAGTATGTAAGATAGTAT (21-mer)</td></tr>
</table>
* Note: Annealing of primers to control DNA


</td></tr>
<tr><td>2</td><td> 
Preparation of reaction mixture</td><td>
2. Add first PCR components into the AccuPower® ProFi Taq PCR PreMix tubes to a total volume of 50 µl. Do not calculate the dried pellet.
• Preparation of reaction mixture
<table>
<tr><th>Components</th><th>Negative</th><th>Positive</th><th>Sample</th></tr>
<tr><td>Template DNA (10 ng/µl)</td><td>-</td><td>2.5 µl</td><td>2.5 µl</td></tr>
<tr><td>1st Forward primer (10 pmol/µl)</td><td>2.5 µl</td><td>2.5 µl</td><td>2.5 µl</td></tr>
<tr><td>1st Reverse primer (10 pmol/µl)</td><td>2.5 µl</td><td>2.5 µl</td><td>2.5 µl</td></tr>
<tr><td>Distilled water</td><td>45 µl</td><td>42.5 µl</td><td>42.5 µl</td></tr>
<tr><td>Total volume</td><td>50 µl</td><td>50 µl</td><td>50 µl</td></tr>
</table>
3. Dissolve the vacuum-dried blue pellet by tapping or pipetting, and briefly spin down.
</td></tr>
<tr><td>3</td><td> 
Incubate reactions in a thermal cycler</td><td>
4. Perform the first PCR reaction under the following conditions.
<table>
<tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr>
<tr><td>Pre-denaturation</td><td>94°C</td><td>5 min</td><td>1 cycle</td></tr>
<tr><td>Denaturation</td><td>94°C</td><td>30 sec</td><td rowspan="3">30 cycles</td></tr>
<tr><td>Annealing</td><td>58°C</td><td>30 sec</td></tr>
<tr><td>Extension</td><td>72°C</td><td>1 min/kb</td></tr>
<tr><td>Final extension</td><td>72°C</td><td>5 min</td><td>1 cycle</td></tr>
</table>
* Note: The length of first PCR product from control DNA of 462 bp is 516 bp.
</td></tr>
</table>

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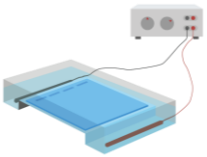

Analyze with gel electrophoresis

5. After the reaction, maintain the reaction mixture at 4-8°C. The samples can be stored at -20°C until use.

6. Load the samples on agarose gel without adding a loading-dye mixture, and perform gel electrophoresis for analysis.

7. Purify the samples using a gel purification kit.

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Preparation of reaction mixture

8. Add second PCR components into the *AccuPower® ProFi Taq* PCR PreMix tubes to a total volume of 50 µl. Do not calculate the dried pellet.

- Preparation of reaction mixture

Components	Negative 1 set 6 PCRs/kit	Positive 1 set 12 PCRs/kit	Sample 5 sets 60 PCRs/kit
Purified 1st PCR products (20 ng/µl)	-	2.5 µl	2.5 µl
Tag 1 (or 2-6)_Cassette mixture	5 µl	5 µl	5 µl
2nd Forward primer (10 pmol/µl)	2.5 µl	2.5 µl	2.5 µl
2nd Reverse primer (10 pmol/µl)	2.5 µl	2.5 µl	2.5 µl
Distilled water	40 µl	37.5 µl	37.5 µl
Total volume	50 µl	50 µl	50 µl

*** Note:** The positive control and the samples require 2 PCR reactions.

9. Dissolve the vacuum-dried blue pellet by tapping or pipetting, and briefly spin down.

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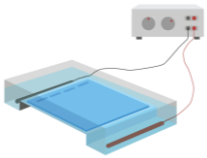
Incubate reactions in a thermal cycler

10. Perform the second PCR reaction under the following conditions.

Step	Temperature	Time	Cycles
Pre-denaturation	94°C	5 min	1 cycle
Denaturation	94°C	1 min	
Annealing	48°C	1 min	33 cycles
Extension	72°C	1 min/kb	
Final extension	72°C	10 min	1 cycle

- Length of each cassette

Cassettes	DNA size	Protein size
Tag 1_Cassette	287 bp	2.2 kDa
Tag 2_Cassette	302 bp	2.9 kDa
Tag 3_Cassette	326 bp	3.8 kDa
Tag 4_Cassette	506 bp	10.5 kDa
Tag 5_Cassette	605 bp	13.7 kDa
Tag 6_Cassette	722 bp	18.8 kDa

7	 **Analyze with gel electrophoresis**	11. After the reaction, maintain the reaction mixture at 4-8°C. The samples can be stored at -20°C until use. 12. Load the samples on agarose gel without adding a loading-dye mixture, and perform gel electrophoresis for analysis. 13. Purify the samples using a PCR purification kit. *** Note:** Each column for purification can be loaded 100 µl of the PCR products.