ExiProgen™ ProXpress PCR Template Kit

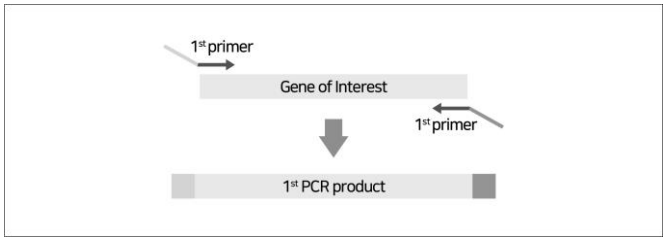
[Cat. No.] K-7400, K-7401, K-7400-CP

Introduction

ExiProgen™ ProXpress PCR Template Kit generates template DNA for cell-free protein expression using a two-step PCR process, with no cloning required. The template DNA includes essential elements such as a T7 promoter, a ribosomal binding site (RBS), a T7 terminator, and a 6x histidine tag for recombinant protein production.

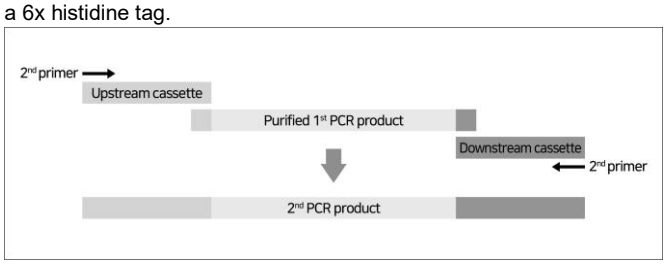
A. First PCR

In the first PCR, target genes are amplified from the DNA (cDNA, genomic DNA, plasmid DNA, etc.) 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 a T7 promoter, a RBS, a T7 terminator, and a 6x histidine tag.



C. Structure of Template DNA

P_{T7} - RBS - Start - 6x His tag	Gene of Interest	Stop - T_{T7}

Figure 1. 6x His-tagged template DNA at the N-terminal

P_{T7} - RBS - Start	Gene of Interest	6x His tag - Stop - T_{T7}

Figure 2. 6x His-tagged template DNA at the C-terminal

Features & Benefits

- 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	K-7400	K-7401	K-7400-CP
AccuPower® *ProFi* Taq PCR Premix	20 µl x 96 tubes	20 µl x 192 tubes	-
N terminus upstream cassette (5 ng/µl)	70 µl (Green, NU)	70 µl x 2 ea (Green, NU)	400 µl
N terminus downstream cassette (5 ng/µl)	70 µl (Green, ND)	70 µl x 2 ea (Green, ND)	400 µl
C terminus upstream cassette (5 ng/µl)	70 µl (Red, CU)	70 µl x 2 ea (Red, CU)	400 µl
C terminus downstream cassette (5 ng/µl)	70 µl (Red, CD)	70 µl x 2 ea (Red, CD)	400 µl
2nd Forward primer (10 pmol/µl)	70 µl (Black, 2F)	70 µl x 2 ea (Black, 2F)	400 µl
2nd Reverse primer (10 pmol/µl)	70 µl (Black, 2R)	70 µl x 2 ea (Black, 2R)	400 µl

*** Note:** For research use only. Not for use in diagnostic or therapeutic procedures.

Specifications

	K-7400	K-7401	K-7400-CP
Reactions	16 rxns	32 rxns	100 rxns
Target DNA size		≤ 1.6 kb	

Storage

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

Online Resources

Korean **English**

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

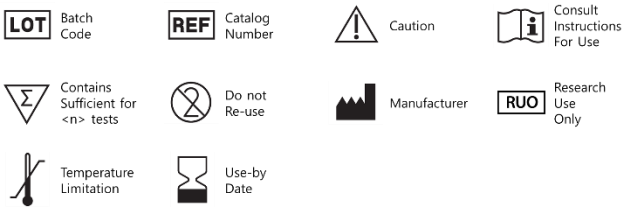
Ordering Information

Description	Reactions	Cat. No.
ExiProgen™ ProXpress PCR Template Kit	16	K-7400
	32	K-7401
	100	K-7400-CP

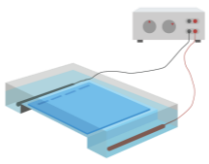
Notice

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. Design and order the gene-specific primers as shown below. The 1st Forward and Reverse primer ordered below is supplied with overlapping sequences (21-mer) to both the upstream and downstream cassettes respectively at the 5’-end of each primer.
<table><tr><th>Primers</th><th>Sequences (5' to 3')</th></tr><tr><td>1st Forward primer (39-mer)</td><td>XXXXXXXXXXXXXXXXXXXX (overlapping on upstream cassette) + 18-mer from the 5’-end of the target gene except for the start codon</td></tr><tr><td>1st Reverse primer (39-mer)</td><td>XXXXXXXXXXXXXXXXXXXX (overlapping on downstream cassette) + 18-mer from the 3’-end of the target gene except for the stop codon in reverse complementary sequence</td></tr></table>* Note: The 1st Forward/Reverse primer sets come in two types, each with Cat. No. N-8229 or N-8230, and which to choose depends on the location of the 6x histidine tag on the template DNA the user wants to synthesize. Refer to the example on our product page for easy understanding.</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 20 µl. Do not calculate the dried pellet.
• Preparation of reaction mixture
<table><tr><th>Components</th><th>Negative</th><th>Sample</th></tr><tr><td>Template DNA (1-500 ng)</td><td>-</td><td>Variable</td></tr><tr><td>1st Forward primer (10 pmol/µl)</td><td>1 µl</td><td>1 µl</td></tr><tr><td>1st Reverse primer (10 pmol/µl)</td><td>1 µl</td><td>1 µl</td></tr><tr><td>Distilled water</td><td>18 µl</td><td>Variable</td></tr><tr><td>Total volume</td><td>20 µl</td><td>20 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> 
First PCR</td><td>4. Perform the first PCR 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></td></tr><tr><td>Annealing</td><td>58°C</td><td>30 sec</td><td>30 cycles</td></tr><tr><td>Extension</td><td>72°C</td><td>1 min/kb</td><td></td></tr><tr><td>Final extension</td><td>72°C</td><td>5 min</td><td>1 cycle</td></tr></table></td></tr>
<tr><td>4</td><td> 
Analyze with gel electrophoresis</td><td>5. Load the samples on the agarose gel without adding a loading-dye mixture, and perform gel electrophoresis for analysis.
* Note: The size of the target product should be 80 bp larger than the size of your target gene due to the first-step primers.
6. Purify the samples using a gel purification kit.</td></tr>
</table>

5

Preparation of reaction mixture

7. Add second PCR components into the *AccuPower® ProFi Taq* PCR PreMix tubes to a total volume of 20 µl. Do not calculate the dried pellet.

- Preparation of reaction mixture

Components	Negative	Sample
Template DNA (Purified 1st PCR product)	-	Variable (>10 ng)
Upstream cassette (5 ng/µl)	1 µl	1 µl
Downstream cassette (5 ng/µl)	1 µl	1 µl
2nd Forward primer (10 pmol/µl)	1 µl	1 µl
2nd Reverse primer (10 pmol/µl)	1 µl	1 µl
Distilled water	16 µl	Variable
Total volume	20 µl	20 µl

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

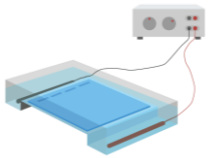
6

Second Overlapping PCR

9. Perform the first PCR 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	30 cycles
Extension	72°C	1 min/kb	
Final extension	72°C	5 min	1 cycle

7



Analyze with gel electrophoresis

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

*** Note:** It is recommended to run the second-step PCR product with the first-step PCR product side-by-side on the same gel. The second-step PCR product will be 200 bp larger than the first-step in size.

11. Purify the samples using a gel purification kit.