

**[Cat. No.]** Please refer to the **Ordering Information**

## Introduction

*Pfu* DNA Polymerase is a thermostable DNA polymerase that originated from *Pyrococcus furiosus*. It catalyzes the polymerization of nucleotides into duplex DNA in the 5' to 3' direction and provides highly accurate PCR products through its proof-reading function, 3' to 5' exonuclease (proofreading) activity. *Pfu* DNA Polymerase is recommended for application of PCR and primer extension reactions that required high fidelity.

## Applications

- Gene synthesis
- PCR or primer extension requested high fidelity
- Blunt-end PCR cloning or mutagenesis requested high fidelity

## Components

| Components                | E-2015            | E-2015-1          | E-2016                  | E-2016-1                |
|---------------------------|-------------------|-------------------|-------------------------|-------------------------|
| <i>Pfu</i> DNA Polymerase | 250 U<br>(100 µl) | 250 U<br>(100 µl) | 1,000 U<br>(100 µl x 4) | 1,000 U<br>(100 µl x 4) |
| 10X Reaction buffer       | 1 ml              | 1 ml              | 1 ml x 4                | 1 ml x 4                |
| 10 mM dNTPs               | -                 | 1 ml              | -                       | 1 ml x 4                |
| Dilution buffer           | 1 ml              | 1 ml              | 1 ml x 4                | 1 ml x 4                |

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

## Specifications

| <i>Pfu</i> DNA Polymerase     |             |
|-------------------------------|-------------|
| 5' to 3' exonuclease activity | No          |
| 3' to 5' exonuclease activity | Yes         |
| 3'-A overhang                 | No          |
| Fragment size                 | Up to 20 kb |

## Buffer Composition

|                     |                                                                                                                                                                               |
|---------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 10X Reaction buffer | 300 mM Tris-HCl, 200 mM KCl,<br>100 mM (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , 0.75% Triton X-100,<br>0.5 mg/ml Acetylated BSA,<br>15 mM MgSO <sub>4</sub> , pH 9.0 |
| Dilution buffer     | 50 mM Tris-HCl, 0.1 mM EDTA, 1 mM<br>DTT, stabilizer, 50% glycerol, pH 8.2                                                                                                    |

## Storage Buffer

*Pfu* DNA Polymerase is supplied in 50% (v/v) glycerol containing 50 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, and stabilizer, pH 8.2.

## Unit Definition

One unit is defined as the amount of enzyme that incorporates 10 nmole of dNTP into acid-insoluble products in 30 min at 72°C.

## Quality Control

- **Nuclease Contamination Assay:** Nuclease activity is not detected after incubation of 1 µg of substrate DNA (supercoiled plasmid and Lambda/*Hind* III DNA) with 5 U of *Pfu* DNA Polymerase in 50 µl reaction volume at 37°C and 70°C for 18 hrs.

## Storage

Store at -20°C. If stored in the recommended temperature, this product will be stable until the expiration date printed out on the label.

## Precautions

- Avoid multiple freeze and thaw cycles and exposure to frequent temperature changes.
- Since the 3' to 5' exonuclease activity of *Pfu* DNA Polymerase may degrade primers, it is critical that it is applied after the addition of dNTPs or at the end.

## Online Resources



Korean



English

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

## Ordering Information

| Description |                     | Cat. No  |
|-------------|---------------------|----------|
| 250 U       | 10X Reaction buffer | E-2015   |
|             | 10 mM dNTP          | E-2015-1 |
| 1,000 U     | 10X Reaction buffer | E-2016   |
|             | 10 mM dNTP          | E-2016-1 |

## 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



Batch Code



Catalog Number



Caution



Consult Instructions For Use



Contains Sufficient for <n> tests



Research Use Only



Temperature Limitation



Use-by Date

## Experimental Procedures

| Steps                                      |                                                                                                                                     | Procedure Details                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
|--------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|---------------------|---------------------|---------------|------------------|----------|-----------------------------------|-------------|---------------|-----------------------------------|-------------|---------------|---------------------|-----------|-----------|--------------|---------------------------|-------------------------|--------------------------------------------|---------|-----------------|---------------------|----------|----------|--------------|------------|------------|
| 1                                          | <br><b>Thaw reagents</b>                           | <p>1. Thaw 10X Reaction buffer and 10 mM dNTPs on ice and mix thoroughly before use. Then, briefly spin down all components including template DNA, <i>Pfu</i> DNA Polymerase, primers and nuclease-free water.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| 2                                          | <br><b>Preparation of reaction mixture</b>         | <p>2. Add all components for PCR into PCR tubes (not provided) to a total volume of 20 <math>\mu</math>l or 50 <math>\mu</math>l.</p> <ul style="list-style-type: none"> <li>Preparation of reaction mixture</li> </ul> <table border="1"> <thead> <tr> <th>Components</th> <th>20 <math>\mu</math>l reaction</th> <th>50 <math>\mu</math>l reaction</th> </tr> </thead> <tbody> <tr> <td>Template DNA*</td> <td>Variable</td> <td>Variable</td> </tr> <tr> <td>Forward primer (10 pmol/<math>\mu</math>l)</td> <td>1-2 <math>\mu</math>l</td> <td>2.5-5 <math>\mu</math>l</td> </tr> <tr> <td>Reverse primer (10 pmol/<math>\mu</math>l)</td> <td>1-2 <math>\mu</math>l</td> <td>2.5-5 <math>\mu</math>l</td> </tr> <tr> <td>10X Reaction buffer</td> <td>2 <math>\mu</math>l</td> <td>5 <math>\mu</math>l</td> </tr> <tr> <td>10 mM dNTPs</td> <td>0.5-2 <math>\mu</math>l or Variable</td> <td>1-5 <math>\mu</math>l or Variable</td> </tr> <tr> <td><i>Pfu</i> DNA Polymerase (2.5 U/<math>\mu</math>l)</td> <td>0.5-1 U</td> <td>1-2.5 U</td> </tr> <tr> <td>Nuclease-free water</td> <td>Variable</td> <td>Variable</td> </tr> <tr> <td>Total volume</td> <td>20 <math>\mu</math>l</td> <td>50 <math>\mu</math>l</td> </tr> </tbody> </table> <p>* Recommended amounts of template DNA is as follows; plasmid and lambda DNA, &gt; 1 pg; bacterial genomic DNA, &gt; 100 pg; human genomic DNA, &gt; 1 ng.</p> <p>3. Mix the reaction mixture by tapping or pipetting, and briefly spin down.</p> | Components   | 20 $\mu$ l reaction | 50 $\mu$ l reaction | Template DNA* | Variable         | Variable | Forward primer (10 pmol/ $\mu$ l) | 1-2 $\mu$ l | 2.5-5 $\mu$ l | Reverse primer (10 pmol/ $\mu$ l) | 1-2 $\mu$ l | 2.5-5 $\mu$ l | 10X Reaction buffer | 2 $\mu$ l | 5 $\mu$ l | 10 mM dNTPs  | 0.5-2 $\mu$ l or Variable | 1-5 $\mu$ l or Variable | <i>Pfu</i> DNA Polymerase (2.5 U/ $\mu$ l) | 0.5-1 U | 1-2.5 U         | Nuclease-free water | Variable | Variable | Total volume | 20 $\mu$ l | 50 $\mu$ l |
| Components                                 | 20 $\mu$ l reaction                                                                                                                 | 50 $\mu$ l reaction                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| Template DNA*                              | Variable                                                                                                                            | Variable                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| Forward primer (10 pmol/ $\mu$ l)          | 1-2 $\mu$ l                                                                                                                         | 2.5-5 $\mu$ l                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| Reverse primer (10 pmol/ $\mu$ l)          | 1-2 $\mu$ l                                                                                                                         | 2.5-5 $\mu$ l                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| 10X Reaction buffer                        | 2 $\mu$ l                                                                                                                           | 5 $\mu$ l                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| 10 mM dNTPs                                | 0.5-2 $\mu$ l or Variable                                                                                                           | 1-5 $\mu$ l or Variable                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| <i>Pfu</i> DNA Polymerase (2.5 U/ $\mu$ l) | 0.5-1 U                                                                                                                             | 1-2.5 U                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| Nuclease-free water                        | Variable                                                                                                                            | Variable                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| Total volume                               | 20 $\mu$ l                                                                                                                          | 50 $\mu$ l                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| 3                                          | <br><b>Incubate reactions in a thermal cycler</b> | <p>4. Perform the reaction under the following conditions.</p> <table border="1"> <thead> <tr> <th>Step</th> <th>Temperature</th> <th>Time</th> <th>Cycles</th> </tr> </thead> <tbody> <tr> <td>Pre-denaturation</td> <td>94°C</td> <td>1 min</td> <td>1 cycle</td> </tr> <tr> <td>Denaturation</td> <td>94°C</td> <td>15-20 sec</td> <td></td> </tr> <tr> <td>Annealing</td> <td>45-65°C†</td> <td>15-30 sec</td> <td>25-35 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>3-5 min</td> <td>1 cycle</td> </tr> </tbody> </table> <p>* <b>Note:</b> For maximum yield and specificity, temperatures and cycling times should be optimized for each new template DNA or primers.<br/> † Set the annealing temperature to 3-5°C lower than the T<sub>m</sub> of the primers.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Step         | Temperature         | Time                | Cycles        | Pre-denaturation | 94°C     | 1 min                             | 1 cycle     | Denaturation  | 94°C                              | 15-20 sec   |               | Annealing           | 45-65°C†  | 15-30 sec | 25-35 cycles | Extension                 | 72°C                    | 1 min/kb                                   |         | Final extension | 72°C                | 3-5 min  | 1 cycle  |              |            |            |
| Step                                       | Temperature                                                                                                                         | Time                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Cycles       |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| Pre-denaturation                           | 94°C                                                                                                                                | 1 min                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 1 cycle      |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| Denaturation                               | 94°C                                                                                                                                | 15-20 sec                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| Annealing                                  | 45-65°C†                                                                                                                            | 15-30 sec                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 25-35 cycles |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| Extension                                  | 72°C                                                                                                                                | 1 min/kb                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| Final extension                            | 72°C                                                                                                                                | 3-5 min                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 1 cycle      |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |
| 4                                          | <br><b>Analyze with gel electrophoresis</b>      | <p>5. After the reaction, maintain the reaction mixture at 4-8°C. The samples can be stored at -20°C until use.</p> <p>6. Load samples on agarose gel with adding a loading-dye mixture, and perform gel electrophoresis for analysis.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |              |                     |                     |               |                  |          |                                   |             |               |                                   |             |               |                     |           |           |              |                           |                         |                                            |         |                 |                     |          |          |              |            |            |