AccuPower® HotStart *Pfu* PCR PreMix

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

Introduction

AccuPower® HotStart *Pfu* PCR PreMix is based on a concept chemical interaction between pyrophosphate (PPi) and pyrophosphatase (PPase). DNA polymerase is required Mg^{2+} to activate, but included PPi binds with high affinities to Mg^{2+} resulting in inhibition of polymerase activity. When the temperature rises during denaturation step, Mg-PPi complex is decomposed into 2Pi and Mg^{2+} by PPase. Then, activated DNA polymerase proceed reactions. This prevents the formation of mis-primed products and primer-dimers during the reaction set up process resulting in improved PCR specificity. Included *Pfu* DNA polymerase provide highly accurate PCR products through its proof-reading function. This product contains vacuum-dried components including *Pfu* DNA Polymerase, dNTPs, reaction buffer, stabilizer, and tracking dye. It simplifies preparation of reaction mixture by adding template DNA and primers without any extra process. After the reaction, samples can be applied directly on agarose gel for analysis.

Applications

- Gene cloning with blunt ends
- Site-directed mutagenesis
- High fidelity amplification
- High specificity PCR
- cDNA amplification

Features & Benefits

- High fidelity: Low mutation rate during DNA amplification due to its high fidelity (error rate = 1.9×10^{-6}).
- Specificity: Minimized non-specific amplification and maximized PCR efficiency by using BIONEER's differentiated PyroHotStart technology.
- User-friendly: Reactants are individually packaged in each of the PCR tubes, it allows any user simply perform PCR by adding template DNA and primers.
- Stability: Included stabilizer enables delivery at room temperature and provides increased stability compared to solution-type products.
- Reproducibility: Mass production under ISO 9001 quality system allows minimized deviation between lots and reproducible results in replicated tests performed under same conditions and variation.

Composition

Composition	Concentration
Pfu DNA Polymerase	1 U
dNTPs (dATP, dCTP, dGTP, dTTP)	Each 250 μM
Reaction Buffer with 1.5 mM $MgCl_2$	1X
Stabilizer and tracking dye	O

Specifications

Pfu DNA Polymerase	
5' to 3' exonuclease activity	No
3' to 5' exonuclease activity	Yes
3'–A overhang	No
Fragment size	Up to 5 kb

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.

Online Resources

Korean **English**

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

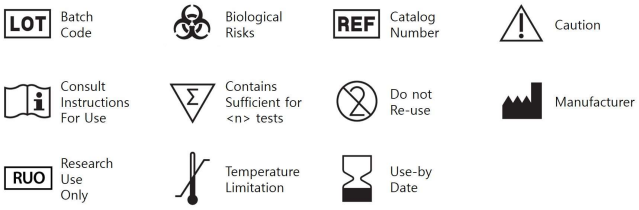
Ordering Information

Description			Cat. No.
0.2 ml thin-wall 8-tube strips with attached cap	96 tubes	20 μl/rxn	K-2301
		50 μl/rxn	K-2302
	480 tubes	20 μl/rxn	K-2303
		50 μl/rxn	K-2304

Notice

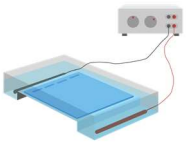
BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Explanation of Symbols



Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td>1</td><td> 
Preparation of reaction mixture</td><td>1. Add template DNA, primers, and nuclease-free water into AccuPower® HotStart Pfu PCR PreMix tubes to make a total volume of 20 µl or 50 µl. Do not include the dried pellet.

• Preparation of reaction mixture
<table><tr><th>Components</th><th>20 µl reaction</th><th>50 µl reaction</th></tr><tr><td>Template DNA (1-500 ng)</td><td>Variable (1-10 µl)</td><td>Variable (1-25 µl)</td></tr><tr><td>Forward primer (10 pmol/µl)</td><td>0.5-2 µl</td><td>1-5 µl</td></tr><tr><td>Reverse primer (10 pmol/µl)</td><td>0.5-2 µl</td><td>1-5 µl</td></tr><tr><td>Nuclease-free water</td><td>Variable</td><td>Variable</td></tr><tr><td>Total volume</td><td>20 µl</td><td>50 µl</td></tr></table>
2. Dissolve the vacuum-dried blue pellet by vortexing or pipetting, and briefly spin down.</td></tr>
<tr><td>2</td><td> 
Incubate reactions in a thermal cycler</td><td>3. Perform the 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>95°C</td><td>5 min</td><td>1 cycle</td></tr><tr><td>Denaturation</td><td>95°C</td><td>30 sec</td><td rowspan="3">30-35 cycles</td></tr><tr><td>Annealing</td><td>55°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: For maximum yield and specificity, temperatures and cycling times should be optimized for each new template DNA or primers.</td></tr>
<tr><td>3</td><td> 
Analyze with gel electrophoresis</td><td>4. After the reaction, maintain the reaction mixture at 4-8°C. The samples can be stored at -20°C until use.

5. Load 5 µl of samples on agarose gel without adding a loading-dye mixture, and perform gel electrophoresis for analysis.</td></tr>
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