AccuPower® PCR PreMix (with UDG)

[Cat. No.] K-2012-1, K-2016-1

Introduction

AccuPower® PCR PreMix (with UDG) helps to minimize carryover contamination, which may cause severe problems in clinical diagnosis by using uracil DNA glycosylase (UDG). UDG catalyzes the hydrolysis of N-glycosylic bond between the uracil and sugar. In the following heating at 95°C, contaminants (uracil-containing DNA) are degraded and consequently not amplified. UDG efficiently remove uracil from single-stranded or double-stranded DNA, but from oligomers (6 or fewer). It is not active for targeting RNA or uracil-free DNA. This product contains vacuum-dried components including UDG, *Top* DNA Polymerase, dNTPs with dUTP, 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.

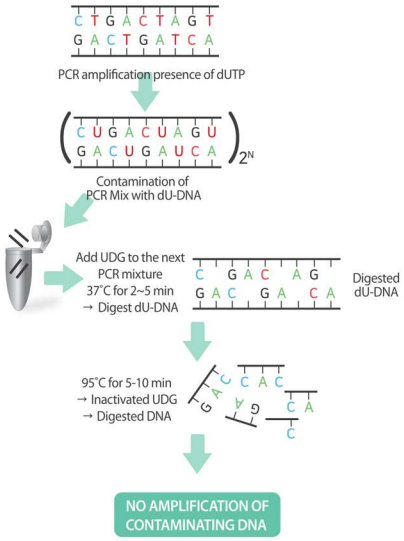


Figure 1. Principle of eliminating contaminants using UDG.

Applications

- Conventional PCR
- Primer extension
- TA cloning
- Gene sequencing
- Molecular diagnosis

Features & Benefits

- Carryover contamination prevention: Minimized false positives caused by a carryover contamination through application of uracil DNA glycosylase system.
- 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 to maintain enzymatic activities for up to 2 years.
- 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
Uracil DNA glycosylase	1 U
Top DNA Polymerase	1 U
dNTPs with dUTP	Each 200 μM
Reaction buffer with 1.5 mM $MgCl_2$	1X
Stabilizer and tracking dye	O

Specifications

Top DNA Polymerase	
5' to 3' exonuclease activity	No
3' to 5' exonuclease activity	No
Terminal transferase activity	Yes
Fragment size	Up to 10 kb

Enzyme Inactivation

UDG is inactivated by heating at 95°C for 5 min.

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

- UDG activities can be remained after finishing reactions, if it is kept on below 50°C. Therefore, reaction mixture is recommended to freeze immediately after the reaction.

Online Resources

Korean **English**

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

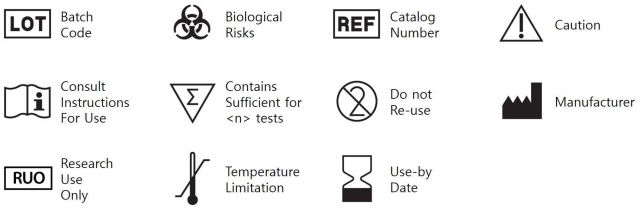
Ordering Information

Description			Cat. No.
0.2 ml thin-wall 8-tube strips with attached cap	96 tubes	20 μl/rxn	K-2012-1
	480 tubes	20 μl/rxn	K-2016-1

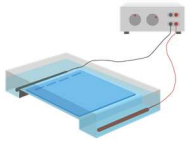
Notice

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



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® PCR PreMix (with UDG) tubes to make a total volume of 20 µl. Do not include the dried pellet.
• Amount of template
<table><tr><th>Template DNA</th><th>Amount of template</th></tr><tr><td>Bacteriophage λ, Plasmid DNA</td><td>100 fg-200 ng</td></tr><tr><td>Total genomic DNA</td><td>1-500 ng</td></tr></table>• Preparation of reaction mixture
<table><tr><th>Components</th><th>20 µl reaction</th></tr><tr><td>Template DNA</td><td>Variable (1-10 µl)</td></tr><tr><td>Forward primer (10 pmol/µl)</td><td>0.5-2 µl</td></tr><tr><td>Reverse primer (10 pmol/µl)</td><td>0.5-2 µl</td></tr><tr><td>Nuclease-free water</td><td>Variable</td></tr><tr><td>Total volume</td><td>20 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>UDG activation</td><td>37°C</td><td>2 min</td><td>1 cycle</td></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>0.5-1 min</td><td rowspan="4">25-35 cycles</td></tr><tr><td>Annealing</td><td>45-65°C*</td><td>0.5-1 min</td></tr><tr><td>Extension</td><td>72°C</td><td>0.5-1 min/kb</td></tr><tr><td>Final extension</td><td>72°C</td><td>3-5 min</td><td>1 cycle</td></tr></table>* Set the annealing temperature to 3-5 degrees lower than the Tm of the primers</td></tr>
<tr><td>3</td><td> 
Analyze with gel electrophoresis</td><td>4. After the reaction, maintain the reaction mixture at -20°C or load samples on agarose gel immediately.
5. Load samples on agarose gel without adding a loading-dye mixture, and perform gel electrophoresis for analysis.</td></tr>
<tr><td></td><td> 
Option</td><td>• If primer’s Tm value is more than 65°C or PCR product size is more than 5 kb, follow the conditions as below.
<table><tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr><tr><td>UDG activation</td><td>37°C</td><td>2 min</td><td>1 cycle</td></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="2">30-35 cycles</td></tr><tr><td>Annealing/Extension</td><td>68°C</td><td>1 min/kb</td></tr><tr><td>Final extension</td><td>72°C</td><td>3-5 min</td><td>1 cycle</td></tr></table></td></tr>
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