AccuPower® HotStart PCR PreMix (with UDG) (V3/2022-02-07)

[Cat. No.] **K-5050-1, K-5051-1**

Introduction

AccuPower® HotStart PCR PreMix (with UDG) is based on a concept chemical interaction between pyrophosphate (PPi) and pyrophosphatase (PPase). 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. This product also 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.

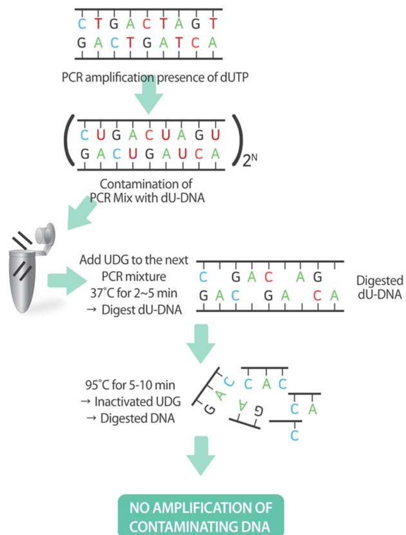


Figure 1. Principle of eliminating contaminants using UDG.

Applications

- gDNA template PCR
- Low-copy target PCR
- Multiple primer pairs PCR
- cDNA template PCR
- 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
HotStart 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

HotStart DNA Polymerase	
5' to 3' exonuclease activity	No
3' to 5' exonuclease activity	No
3'–A overhang	Yes
Fragment size	Up to 12 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

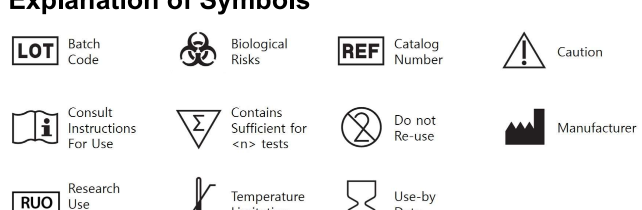
Ordering Information

Description			Cat. No.
0.2 ml thin-wall 8-tube strips with attached cap	96 tubes	20 μl/rxn	K-5050-1
	480 tubes	20 μl/rxn	K-5051-1

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



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Experimental Procedures

Steps		Procedure Details
1	Preparation of reaction mixture	1. Add template DNA, primers, and nuclease-free water into *AccuPower*® HotStart PCR PreMix (with UDG) tubes to make a total volume of 20 µl. Do not calculate the dried pellet.
2	Incubate reactions in a thermal cycler	3. Perform the reaction under the following conditions.
3	Analyze with gel electrophoresis	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.

Step 1 details:

- Amount of template

Template DNA	Amount of template
Bacteriophage λ, Plasmid DNA	100 fg-100 ng
Total genomic DNA	1-100 ng

- Preparation of reaction mixture

Components	20 µl reaction
Template DNA	Variable (1-10 µl)
Forward primer (10 pmol/µl)	0.5-2 µl
Reverse primer (10 pmol/µl)	0.5-2 µl
Nuclease-free water	Variable
Total volume	20 µl

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

Step 2 details:

3. Perform the reaction under the following conditions.

Step	Temperature	Time	Cycles
UDG activation	37°C	2 min	1 cycle
Pre-denaturation	95°C	5 min	1 cycle
Denaturation	95°C	0.5-1 min	25-35 cycles
Annealing	50-65°C*	0.5-1 min	
Extension	72°C	0.5-1 min/kb	
Final extension	72°C	3-5 min	1 cycle

* Set the annealing temperature to 3-5 degrees lower than the Tm of the primers.

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