AccuPower® HotStart PCR PreMix (with UDG)

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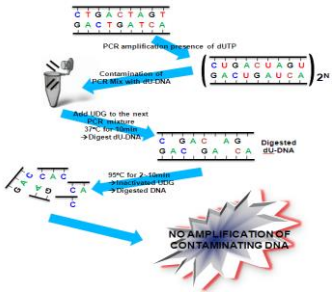
I. Introduction

AccuPower® HotStart PCR PreMix (with UDG) was designed by Chemical-Mediated Hotstart method. The DNA polymerase is inhibited by the pyrophosphate, but activated upon pyrophosphate hydrolysis by the thermostable pyrophosphatase (Patent pending). This prevents the formation of mis-primed products and primer-dimers during the reaction set up process resulting in improved PCR specificity. At the same time, *AccuPower*® HotStart PCR PreMix (with UDG) contains uracil DNA glycosylase can be resolved.

AccuPower® HotStart PCR PreMix (with UDG) contains uracil DNA glycosylase, dA, dG, dC, dTTP with dUTP and reaction buffer in a premixed format that is vacuum-dried into individual tubes.

Carryover contamination is a significant source of error when PCR is being used in a diagnostic context. Use of uracil DNA glycosylase is a solution for this problem which catalyze the hydrolysis of N-glycosylic bond between the uracil and sugar. Heating at 94℃ lead to degrade contaminants (uracil-containing DNA) of PCR reaction mixture. 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 contaminant using UDG



II. Application

AccuPower® HotStart PCR PreMix (with UDG) is recommended for use in Polymerase Chain Reaction (PCR), primer extension reactions, Gene Synthesis, Gene cloning, site-directed mutagenesis and overcome carryover contamination.

III. Storage

AccuPower® HotStart PCR PreMix (with UDG) should be stored at –20℃ upon received, and are stable until the expiry date stated on the label.

IV. Caution

Note that UDG can remains their activity after finishing DNA synthesis, if it is kept on below 50°C. Therefore PCR product is recommended to freeze immediately after PCR reaction.

V. Enzyme inactivation.

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

VI. Product components

Components	Volume
Uracil DNA glycosylase	1 U
Top DNA polymerase	1 U
dNTP with dUTP (each)	200 μM

VII. Protocol

This protocol serves only as a guideline for PCR amplification. Optimal reaction conditions such as incubation time, temperature and amount of template DNA must be individually determined.

1. Add template DNA and primers into *AccuPower*® HotStart PCR PreMix (with UDG) tubes.
2. Add distilled water into *AccuPower*® HotStart PCR PreMix (with UDG) tubes to a total volume of 20 μl. Do not calculate the dried pellet.

Components	Volume
Template DNA	Variable (1~10)
Primer (10 pmole/μl)	0.5~2 μl
D.W.	Variable (6~18 μl)
Total volume	20 μl

▶Note : Amount of template

Template DNA	Amount of template
Bacteriophage λ, Plasmid DNA	100 fg~100 ng
Total Genomic DNA	1 ng~100 ng

3. Dissolve the vacuum-dried blue pellet by vortexing, and briefly spin down.
4. 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	30 sec~1 min	25~35 cycles
Annealing	50~65°C	30 sec~1 min	
Extension	72°C	30 sec ~ 1 min/kb	
Final Extension	72°C	Optional Normally 3~5 min	1 cycle

5. PCR product is recommended to freeze immediately after PCR reaction.
6. Load samples on agarose gel without adding a loading-dye mixture and perform electrophoresis.

VIII. Ordering Information.

Cat. No.	Product
K-5050-1	*AccuPower*® HotStart PCR PreMix (with UDG), 0.2 ml thin-wall 8-strip tubes with attached cap / 96 tubes, 20 μl reaction
K-5051-1	*AccuPower*® HotStart PCR PreMix (with UDG), 0.2 ml thin-wall 8-strip tubes with attached cap / 480 tubes, 20 μl reaction

IX. Notice

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