HotStart DNA Polymerase

[Cat. No.] E-3150, E-3150-1, E-3151

Introduction

HotStart DNA Polymerase was designed by Chemical-Mediated Hotstart method. The DNA polymerase is inhibited by the pyrophosphate, but activated upon pyrophosphate hydrolysis by the thermostable pyro-phosphatase (Patent pending). This prevents the formation of mis-primed products and primer-dimers during the reaction set up process resulting in improved PCR specificity. In addition, HotStart DNA Polymerase needs not to be activation step.

Applications

- HotStart PCR, PCR with complex genomic templates/low copy templates/cDNA
- Multiplex PCR
- Primer extension
- SNP typing
- Real-time PCR using dsDNA binding dye
- Multiple primer pairs and amplification of low copy template DNA

Components

Components	E-3150	E-3150-1	E-3151
HotStart DNA Polymerase	250 U (50 µl)	250 U (50 µl)	1,000 U (50 µl x 4)
10X Reaction buffer	0.5 ml	0.5 ml	0.5 ml x 4
10 mM dNTPs	0.5 ml	-	0.5 ml x 4
20 mM $MgCl_2$	0.5 ml	0.5 ml	0.5 ml x 4
Dilution buffer	0.5 ml	0.5 ml	0.5 ml x 4

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

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

*** Note:** This enzyme is specifically optimized for increasing base incorporation rate by inactivating 5' to 3' exonuclease activity. Therefore, this product is not recommended to use for real-time PCR using TaqMan probe.

Buffer Composition

10X Reaction buffer	100 mM Tris-HCl, 400 mM KCl, 20 mM Pyro-phosphate, pH 9.0
Dilution buffer	50 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, stabilizer, 50% glycerol, pH 8.2

Storage Buffer

HotStart DNA Polymerase is supplied in 50% (v/v) glycerol containing 50 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, and stabilizer, pH 8.0.

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 HotStart 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.

Online Resources

Korean **English**

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

Ordering Information

Description			Cat. No
250 U	10X Reaction buffer, 20 mM $MgCl_2$	10 mM dNTPs	E-3150
			E-3150-1
1,000 U	10X Reaction buffer, 20 mM $MgCl_2$	10 mM dNTPs	E-3151

Notice

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Explanation of Symbols

Experimental Procedures

Step 1 — Thaw reagents

1. Thaw 10X Reaction buffer, 10 mM dNTPs, and 20 mM $MgCl_2$ on ice and mix thoroughly before use. Then, briefly spin down all components including template DNA, HotStart DNA Polymerase, primers and nuclease-free water.

Step 2 — Preparation of reaction mixture

2. Add all components for PCR into PCR tubes (not provided) to a total volume of 20 µl or 50 µl.

- Preparation of reaction mixture

Components	20 µl reaction	50 µl reaction
Template DNA*	Variable	Variable
Forward primer (5 pmol/µl)	1-2 µl	2-5 µl
Reverse primer (5 pmol/µl)	1-2 µl	2-5 µl
10X Reaction buffer	2 µl	5 µl
10 mM dNTPs	2 µl or Variable	5 µl or Variable
20 mM $MgCl_2$†	Variable	Variable
HotStart DNA Polymerase (5 U/µl)	0.5-1 U	1-2.5 U
Nuclease-free water	Variable	Variable
Total volume	20 µl	50 µl

* Recommended amounts of template DNA is as follows; plasmid and lambda DNA, > 1 pg; bacterial genomic DNA, > 10 pg; human genomic DNA, > 1 ng.
† Recommended final concentration of $MgCl_2$ is 1.5-2 mM.

3. Mix the reaction mixture by tapping or pipetting, and briefly spin down.

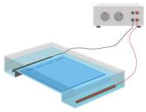
Step 3 — Incubate reactions in a thermal cycler

4. Perform the reaction under the following conditions.

Step	Temperature	Time	Cycles
Pre-denaturation	95°C	5 min	1 cycle
Denaturation	95°C	0.5-1 min	
Annealing	45-65°C†	0.5-1 min	25-35 cycles
Extension	72°C	1 min/kb	
Final extension	72°C	5-10 min	1 cycle

* **Note:** For maximum yield and specificity, temperatures and cycling times should be optimized for each new template DNA or primers.
† Set the annealing temperature to 3-5°C lower than the Tm of the primers.

Step 4 — Analyze with gel electrophoresis



5. After the reaction, maintain the reaction mixture at 4-8°C. The samples can be stored at -20°C until use.

6. Load samples on agarose gel with adding a loading-dye mixture, and perform gel electrophoresis for analysis.