HotStart DNA Polymerase

Cat.No. : E-3150
Lot No. :

● **Description :**

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 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. In addition, HotStart DNA polymerase needs not to be activation step.

● **Unit definition :**

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 minutes at 72°C.

● **Applications :**

HotStart DNA polymerase is recommended for use in Polymerase Chain Reaction(PCR), Primer extension, SNP typing, Multiplex PCR, Real-Time PCR excepting Taqman® probe, multiple primer pairs and amplification of low copy number targets.

● **Notice to purchaser :**

This enzyme is specifically optimized for increasing base incorporation rate by inactivating 5'→3' exonuclease activity. Therefore, this is not recommended to use for Real Time PCR using Taqman® probe, which will be released soon.

● **Concentration :**

5 units/ul

● **Supplied with :**

10X Reaction Buffer : Contains Tris-HCl, KCl, Pyrophosphate, pH 9.0
Enzyme dilution buffer : 50 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, Stabilizers, 50 % Glycerol, pH 8.2.
dNTPs mixture : 10 mM, each dNTP 2.5 mM
20 mM $MgCl_2$

● **Storage condition :**

HotStart DNA polymerase, including buffers and reagents, should be stored immediately upon receipt at -20 °C in a constant temperature freezer.

● **Quality Assurance :**

Nuclease activity is not detected after incubation of 1 ug of substrate DNA – supercoiled plasmid and lambda/*Hin*d III DNA - with 5 units of HotStart DNA Polymerase in 50 uL reaction volume with the supplied Reaction buffer for 18 hr at 37 °C and 70 °C.

● **General Reaction Condition**

Reagent \ Reaction volume	20 uL	50 uL
Template	variable	Variable
Forward primer	5~10 pmoles	10~25 pmole
Reverse primer	5~10 pmoles	10~25 pmole
10X reaction buffer	2 uL	5 uL
10 mM dNTP mixture	2 uL	5 uL
20 mM $MgCl_2$	Variable	Variable
HotStart DNA polymerase	0.5 ~ 1.0 unit	1 ~ 2.5 unit
Distilled Water	Variable	Variable

* Amounts of template
- Plasmid and lambda DNA → more than 1 pg
- Bacterial genomic DNA → more than 10 pg
- Human genomic DNA → more than 1ng

* Concentration of $MgCl_2$
- Final concentration become 1.5 mM ~ 2 mM using 20 mM $MgCl_2$

● **Recommended Cycling parameter**

Step	Temperature	time	Number of cycles
Pre-denaturation	95 °C	5 min	1 cycle
Denaturation	94 °C	0.5~1 min	25~35 cycle
Annealing	45 ~ 65 °C	0.5~1 min	
Extension	72 °C	1 min/ kb	
Final extension	72 °C	5~10 min	1 cycle

Use PCR conditions optimized for *Top* DNA polymerase. In the case of low amount of DNA template, additional cycles may be used.

Note : For research use only. DO NOT use for diagnostic or therapeutic procedures.