

HS Prime Tag DNA Polymerase

Cat. No.	Size	Remark
G-7000	250 units	with dNTPs mix., +Mg buffer

Package Information

G-7000	 HS Prime Taq DNA Polymerae (2.5 units/µℓ): 100 µℓ 10x reaction buffer (with MgCl₂): 1.0 mL 10mM dNTP Mixture (2.5mM of each dNTPs): 0.5 mL
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Description

HS Prime Taq DNA Polymerase is designed for hot-start PCR, a technique that enhances the specificity, sensitivity and yield of DNA amplification. In addition, the enzyme provides the convenience of reaction set-up at room temperature. The enzyme is inactived at room temperature, avoiding extension of non-specifically annealed primers or primer dimers and providing higher specificity of DNA amplification. The functional activity of the enzyme is restored during 10 minute incubation at 94°C. The activated enzyme maintains the same functionality as Taq DNA Polymerase.: it catalyzes $5' \rightarrow 3'$ synthesis of DNA, has no detectable $3' \rightarrow 5'$ proofreading exonuclease activity.

Buffer and Reagents

Storage Buffer: 20 mM Tris-HCl (pH 8.0), 100 mM KCl,

0.5 mM EDTA, 0.1 mM DTT, 0.5% Tween 20 and 50% Glycerol

10x Reaction Buffer: Contains Tris-HCl (pH 9.0), 20 mM MgCl₂, (NH₄)₂SO₄ and PCR enhancers.

10mM dNTP mixture: 2.5mM of each dNTPs

Applications

Hot-start PCR, RT-PCR, Amplification of low copy or high range size DNA target, Real-time PCR, Multiplex PCR and T-vector cloning.

Protocol

Optimal reaction conditions, such as reaction time, temperature and amount of template DNA, may vary and must be individually determined.

1. Thaw 10x reaction buffer and others components.

2. Prepare a master mix.

Components	Volume	Final Conc.
10x reaction buffer	5 µl	1x
10mM dNTP Mix.	1 ~ 5µl	0.2 ~ 1.0 mM
25mM MgCl ₂ ⁽¹⁾	2 ~ 10µl	1 ~ 5 mM
Upstream Primer	Variable	0.1 ~ 1.0 uM
Downstream Primer	Variable	0.1 ~ 1.0 uM
HS Prime Taq DNA Pol. (2.5u/µl)	0.5 ~ 1µl	0.5 ~ 1.0 u
Template DNA ⁽²⁾	Variable	10 fg~1 μg
Sterilized D.W	Up to 50 <i>µ</i> ℓ	-
Total Volume	50 µl	-

(1) If user use the 10x reaction buffer(-Mg), user can select MgCl₂ conc.. (2) Amount of template:

- Bacteriophage lambda, cosmid, plasmid: 10 fg ~ 300 ng

- Total genomic DNA: 100 ng ~ 1 ug

3. Mix the master mix and dispense appropriate volumes into PCR tubes. Centrifuge the reactions in a micro-centrifuge for 10 seconds.

4. Perform PCR using your standard parameters (3-step cycling).

C:	Temp.		
Step	Temp.	Time	Cycles
Initial denaturation	95℃	5~10 min.	1
Amplification	95℃ X℃ 72℃	30 sec. 30 sec. 30~60 sec.	25 ~ 35
Final extension	72℃	5 min.	1

* For PCR products longer than 3~4kb, use an extension time of approximately 1 min per Kb DNA.

5. Separate the PCR products by agarose gel electrophoresis and visualize with EtBr or any other means.

* A DNA fragment which is amplified by HS Prime Taq DNA Polymerase has A-overhang, and it enables you to do cloning by using T-vector.

Research Use Only

Store at -20°C

(Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.)

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