Optimus™ Hotstart Taq DNA Polymerase

Catalog No: P1021 (250U) P1022 (1,000U)

Components

Component	P1021	P1022
Optimus™ Hotstart Taq DNA Polymerase (5U/ul)	50 µl	200 µl
10x Hotstart Buffer(Mg^{2+} Plus)	1.25 ml	1.25 ml x 2
6x Loading Buffer	1 ml	1 ml

Storage

2 years at -20°C

Description

Optimus™ Hotstart Taq DNA Polymerase is a hot-start polymerase with chemical modification, which brings higher specificity by reducing non-specific products as the enzyme activity is temperature-dependent and is inhibited at room temperature. The amplification length and speed can reach to 5 kb (simple template) and 2min/kb (simple template up to 20s/kb) separately. Hotstart Taq has 5'-3' polymerase activity, but no 3'-5' exonuclease activity. The product of Hotstart Taq has overhanged dA at 3'-end. Optimus™ Hotstart Taq DNA polymerase has zero animal source pollution by being produced with advanced chemical modification. And it is much more stable than antibody-modified hot-start polymerase. Its efficiency is higher than most chemical-modified polymerase and the initial-denaturation time can be reduced to 3 minutes.

Optimus™ Hotstart Taq DNA polymerase is an innovative and useful product.

Unit Definition

One enzyme unit (U) refers to the amount of enzyme needed for intaking 10 nmol nucleotides when using activated salmon sperm DNA as template/primer, at 72 °C, in 30 minutes

Quality control

The absence of endonuclease or exonuclease is confirmed by appropriate quality tests. PCR detects no host residual DNA, and it can effectively amplify the single-copy gene of human genome. There is no significant change about the amplification activity after one week at room temperature.

Storage Buffer

200 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5% Tween 20, 50% Glycerol

0.5% Triton X -100

10× Hotstart Buffer with Mg^{2+}

50 mM KCl, 100 mM Tris-HCl, 200 mM NH_4Cl, 20 mM $MgCl_2$

Basic PCR Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation time and temperature, concentration of Taq DNA Polymerase, primers, Mg2+, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile microcentrifuge tube

1.1 Recommended PCR assay with PCR Buffer (Mg2+ plus)

For a 50 µl reaction volume:

Component	Volume	Final concentration
10× Hotstart Buffer (Mg^{2+} plus)	5 µl	1×
dNTPs （2.5mM each）	4 µl	0.2 mM each
Forward primer (10 µM)	2 µl	0.4 µM
Reverse primer (10 µM)	2 µl	0.4 µM
Optimus™ Hotstart Taq DNA Polymerase （5U/µl）	0.5-1 µl	2.5-5U
Template DNA	variable	100 pg-1 µg
Water, nuclease-free	to 50 µl	–

1.2 Recommended PCR assay with PCR Buffer (Mg^{2+} free)

For a 50 µl reaction volume:

Component	Volume	Final concentration
10× Hotstart Buffer (Mg^{2+} free)	5 µl	1×
dNTPs （2.5 mM each）	4 µl	0.2 mM each
Forward primer (10 µM)	2 µl	0.4 µM
Reverse primer (10 µM)	2 µl	0.4 µM
25mM Mg^{2+}	variable	1-4 mM
Optimus™ Hotstart Taq DNA Polymerase （5U/µl）	0.5-1 µl	2.5-5U

Template DNA	variable	100 pg-1 µg
Water, nuclease-free	to 50 µl	–

Table for selection of 25 mM $MgCl_2$ solution volume in 50µl reaction mix:

Final Mg^{2+} conc.	1.0 mM	1.5 mM	2.0 mM	2.5 mM	3 mM	4 mM
Mg^{2+} Stock	2 µl	3 µl	4 µl	5 µl	6 µl	8 µl

Recommendations with Template DNA in a 50 µl reaction volume:

Human genomic DNA	0.1 µg-1 µg
Plasmid DNA	0.5 ng-5 ng
Phage DNA	0.1 ng-10 ng
E.coli genomic DNA	10 ng-100 ng

2. Mix contents in the tube. Seal tubes and centrifuge briefly to collect the contents to the bottom.

When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µl mineral oil.

3. Perform PCR amplification as follows:

Stage	Temperature	Time	Cycles
Initial Denaturation	94°C	3 min	1
Denaturation	94°C	30 s	25-35
Annealing	55-68°C	30 s	
Extension	72°C	1-2 min	
Final Extension	72°C	10 min	1

Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.

4. Analyze the amplification products by agarose gel electrophoresis and visualize by nucleic acid staining. Use appropriate molecular weight standards.

Notes

1 Optimus™ Hotstart Taq DNA Polymerase adopts improved chemical modification technology, so it relies on temperature to activate the polymerase activity, which can effectively inhibit non-specific bindings, and the reaction system can be configured at room temperature.

2 Optimus™ Hotstart Taq DNA Polymerase has the deoxidizing nucleotide transfer activity, so the 3'-end of the PCR product is usually added an extra deoxygenated adenosine nucleoside (dA).

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 10U Optimus™ Hotstart Taq DNA Polymerase with 1µg pBR322 DNA for 4 hours at 37°C and 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 10U Optimus™ Hotstart Taq DNA Polymerase with 1µg digested DNA for 4 hours at 37°C and 70°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10U Optimus™ Hotstart Taq DNA Polymerase with 1µg E.coli [3H]-RNA (40000cpm/µg) for 4 hours at 37°C and 70°C.

Q&A

Why the specificity of Optimus™ Hotstart Taq DNA Polymerase is higher than ordinary Taq DNA Polymerase?

The Optimus™ Hotstart Taq DNA Polymerase is a chemical modification technique that modifies the ordinary Taq DNA Polymerase, which activity is completely inhibited at room temperature and relies on temperature to activate. At low temperature, the enzyme activity is inhibited, but it is activated at high temperature in a very short time. Low temperature inhibition & high temperature activation, so the specificity is better.

Does the product of Optimus™ Hotstart Taq DNA polymerase can be used for TA cloning directly?

Yes. Because the product has 3'-dA end.