Optimus™ Hotstart Mix

Cat No.	P1031	1 ml
	P1032	5 ml
	P1033	100 ml
	P1034	500 ml

Components

Component	P1031	P1032	P1033	P1034
2× Optimus™ Hotstart Mix	1 ml	1 ml × 5	100 ml	500 ml
Nuclease-free water	1 ml	1 ml × 5	-	-

Storage

2 years at -20°C.

Description

2× Optimus™ Hotstart Mix is a premixed, ready-to-use solution containing Hotstart Taq DNA Polymerase, dNTPs, Mg^{2+} and Reaction Buffer at optimal concentrations for efficient amplification of DNA templates by PCR. To prepare the final PCR, only need to add primers and template DNA. This premixed formulation saves time and reduces contamination due to the fewer pipetting steps required for PCR set-up. The mix retains all features of Hotstart Taq DNA Polymerase.

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 polymerases and the initial-denaturation time can be reduced to 3 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.

Composition of the 2× Optimus™ Hotstart Mix

0.5U/ul Optimus™ Hotstart Taq DNA Polymerase, 2× PCR buffer, 0.4mM dNTPs, 3.2 mM $MgCl_2$, 0.02% bromophenol blue.

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 Hotstart Taq DNA Polymerase, primers, Mg^{2+}, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile microcentrifuge tube

For a 50 µl reaction volume:

Component	Volume	Final concentration
Optimus™ Hotstart Mix (2×)	25 µl	1×
Forward Primer	variable	0.1-1 µM
Reverse Primer	variable	0.1-1 µM
Template DNA	variable	10 pg-1 µg
Water, nuclease-free	to 50 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 Mix can be used for TA cloning directly?

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