HS Hotstart Taq DNA Polymerase

For research use only

Components

Component	P1091 (500 U)
10 × HS Hotstart Taq Buffer (Mg^{2+} plus)	1 ml × 2
dNTP Mix (10 mM each)	400 µl
HS Hotstart Taq DNA Polymerase (5 U/µl)	100 µl

Storage

This reagent should be kept at -30 ~ -15°C.

Description

HS Hotstart Taq DNA Polymerase is a hot-start Taq DNA polymerase produced by mixing Taq antibody with Taq DNA polymerase in optimum proportion. Based on the thermostable properties of Taq antibody, the activity of HS Hotstart Taq DNA Polymerase remains strictly closed at 55°C, minimizing non-specific amplification during the mixing and heating phases of the reaction system. When the reaction is maintained above 30 sec at 95°C, Taq antibody is completely inactivated and the polymerase activity is fully released, ensuring the extremely high amplification sensitivity and specificity of the PCR system. The activation of HS Hotstart Taq DNA Polymerase is not affected by buffer pH, ion strength or other factors. It is suitable for various hot-start PCR and qPCR reactions based on Taq DNA polymerase. It can be used to amplify low-copy genes from complicated templates (genome, cDNA) and is the preferred hot-start Taq DNA polymerase for molecular diagnostic reagents based on PCR/qPCR. The elongation rate is 1kb/min. The PCR product has 3 '-d A ends and can be cloned to T vector.

Unit Definition

Using activated salmon sperm DNA as template/primer, the activity of ingesting 10 nmol total nucleotides as acid insoluble substances within 30 min at 74°C is defined as 1 unit of activity (U).

Quality Control

Detection of closure of activity: in 1 × HS Hotstart Taq Buffer, the reaction was conducted at 65°C for 30 min, and the released activity was < 5%.

Detection of release of activity: in 1 × HS Hotstart Taq Buffer, after heating at 95°C for 30 sec, and the released activity was > 95%.

Exonuclease residue detection: 50 U of this polymerase and 50 pmol ssDNA and dsDNA substrate were incubated at 37°C for 16 h, and the DNA bands did not change after denaturation PAGE.

Detection of endonuclease residue: 50 U of this polymerase and 0.3 µg of pBR322 DNA were incubated at 37°C for 4 h, and the DNA bands of the plasmids were not changed by agarose gel electrophoresis.

RNase residue detection: 50 U of this polymerase and 1 µg of 293 cell RNA were incubated at 37°C for 30 min, and the RNA bands was unchanged by agarose gel electrophoresis.

E.coli DNA residue detection: the nucleic acid residue in 50 U of this polymerase was detected by *E. coli* gDNA-specific TaqMan qPCR, and the residue of *E. coli* genome was less than 10 copies.

Functional detection: In the 20 µl PCR system, cDNA corresponding

to the total RNA of 1 pg-200 ng HeLa cells was used as the template to amplify 4 different gene loci. The gradient of PCR product yield could be detected by fluorescence after 35 cycles. At a minimum, the corresponding amplification products were obtained from cDNA corresponding to the total RNA of 1 pg HeLa cells.

Protocol

Reaction system

Component	Amount
10 × HS Hotstart Taq Buffer (Mg^{2+} plus)[a]	5 μl
dNTP Mix (10 mM each)	1 μl
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
Template DNA[b]	x μl
HS Hotstart Taq DNA Polymerase (5 U/μl)[c]	0.5 μl
ddH_2O	to 50 μl

a. For most PCR reactions, the optimal final concentration of Mg^{2+} is 1.5-2 mM. Mg^{2+} with a final concentration of 2 mM has been included in the system. If necessary, the optimal concentration of Mg^{2+} can be explored with 25 mM $MgCl_2$ at intervals of 0.2-0.5 mM.

b. The optimal reaction concentration of different templates varies, and the table below shows the recommended amount of 50 μl reaction system templates.

Human genome DNA	1-500 ng
E.coli genome DNA	1-100 ng
λ DNA	0.1-10 ng
Plasmid DNA	0.1-10 ng

c. The amount of enzyme can be adjusted between 0.125-0.5 μl. In general, increasing the amount of enzyme can increase the amplification yield, but it may decrease the specificity.

* When the GC content of the amplified fragment is > 60%, and normal amplification cannot be achieved after optimizing the conditions, PCR Enhancer (DSBio #P9041) is recommended to optimize PCR reaction.

Reaction procedure

Temperature	Time	Cycle
95°C	30 sec (initial denaturation)	/
95°C	30 sec	30~35
55°C*	30 sec	
72°C	60 sec/kb	
72°C	7 min (final extension)	/

* The annealing temperature should be adjusted according to the Tm value of the primer, which is generally set to be 3-5°C lower than the Tm value of the primer.

Matters Needing Attention

Primer design

1. The last base at the 3 'end of the primer should be G or C;
2. The last 8 bases at the 3 'end of the primer should avoid continuous mismatch;
3. Hairpin structure should be avoided at the 3 'end of primer;
4. The difference of Tm value between the forward Primer and the reverse Primer is better less than 1°C, and the Tm value is better when adjusted to 55-65°C (Primer Premier 5 is recommended for Tm value calculation).
5. Additional primer sequence, that is, unpaired sequence with the

template, should not be involved in the calculation of primer Tm value;

6. GC content of primer is controlled between 40-60%;

7. The overall distribution of bases A, G, C and T should be as uniform as possible, and avoid areas with high GC or AT content.

8. Avoid complementary sequences with more than 5 bases in the primer or between the two primers, and avoid complementary sequences with more than 3 bases at the 3 'end of the two primers;

9. After primer design, please use NCBI BLAST function to retrieve primer specificity to avoid non-specific amplification.