HS™ Taq DNA Polymerase

Ref : NB-03-0103 250 U
NB-03-0104 500 U
NB-03-0105 1 000 U
NB-03-0106 18 000 U

Contents

	NB-03-0103	NB-03-0104	NB-03-0105	NB-03-0106
HS™ Taq DNA Polymerase (5U/µl)	50 µl	100 µl	200 µl	100 µl x36
10X HS™ PCR Buffer	1.4 ml	1.4 ml	1.4 ml x2	1.4 ml x36
6X Loading Buffer	1 ml	1 ml	1 ml	--

Description

HS™ Taq DNA Polymerase is a thermostable recombinant DNA polymerase derived from thermophilic bacterium Thermus aquaticus , its molecular weight is 94 kDa. HS™ Taq DNA Polymerase can amplify DNA target up to 5 kb. The elongation velocity is 0.9~1.2kb/min. It has 5' to 3' polymerase activity but lacks of 3' to 5' exoneclease activity, which results in a 3'-dA overhangs PCR product. All conponents of the HS™ PCR Buffer are at optimal concentration for efficient amplification, it contributes to highly specific incorporation of primer and template.

Features

- Highly thermostable -have a half-life of over 40 min at 95°C incubation
- Generates 3'-dA overhangs PCR products

Applications

- PCRamplification of DNA fragments as long as 5 kb
- DNA labeling
- DNA sequencing
- Generate PCR product for TA cloning

Neo-Biotech
183, avenue Georges Clémenceau – 92000 Nanterre

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases confirmed by appropriate quality tests.
Functionally tested in PCR

10X *HS*™ PCR Buffer

120 mM Tris-HCl (pH 8.8), 500 mM KCl, 1%Triton-X-100, 100 mM Lycine, 25 mM $MgCl_2$

Storage Buffer

20 mM Tris-HCl (pH8.0), 100mM KCl, 3 mM $MgCl_2$ 1mM DTT， 0.1% NP-40, 0.1% Tween20, 0.2mg/ml BSA, 50% (v/v) glycerol

Definition of Activity Unit

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nM of dNTPs into an acid-insoluble form in 30 minutes at 70°C using hering sperm DNA as substrate.

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 HS™ Taq DNA Polymerase primers, $MgCl_2$, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile microcentrifuge tube sitting on ice:
Recommended PCR assay with HS™ PCR Buffer (Mg^{2+} plus)

Reagent	Quantity for 50µl of reaction mixture	Final Concentration
Sterile deionized water	variable	-
10X HS™ PCR Buffer (Mg^{2+} plus)	5 µl	1X
dNTPs (10mM each）	1 µl	0.2 mM each
Primer I	variable	0.4 - 1 µM
Primer II	variable	0.4 - 1 µM
HS™ Taq DNA Polymerase (5U/µl)	0.25 - 0.5 µl	1.25 - 2.5U/50 µl
Template DNA	variable	10pg-1µg
Total		50 µl

Recommandations 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 of tube. Cap 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 25-35 cycles of PCR amplification as follows:

Initial Denaturation	94℃	3 minutes
25-35 cycles	94℃ 55-68℃ 72℃	30 seconds 30 seconds 1 minute
Final extension	72℃	10 minutes

4. 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.

5. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.

Note:

- *HS*™ Taq DNA Polymerase is for High Specificity PCR applications.
- The half-life of enzyme is >40 minutes at 95°C.
- The error rate of *HS*™ Taq DNA Polymerase in PCR is 2.2×10^{-5} errors per nt per cycle; the accuracy (an inverse of the error rate) an average number of correct nucleotides incorporated before making an error, is 4.5×10^{-4} (determined according to the modified method described in).
- *HS*™ Taq DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.

Store all components at –20°C