HS™ Mix

#P2081, 1 ml

Contents:

2× *HS*™ Mix	1 ml
Nuclease-free water	1 ml

Store at -20°C

For research use only.

In total 2 vials.

Description

2× *HS*™ Mix is a premixed, ready-to-use solution containing hotstart *HS*™ Taq DNA Polymerase with antibody modification, 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. *HS*™ Mix contributes to highly reproducible PCR by reducing the risk of pipetting errors, miscalculation and contamination. It also contributes to higher specificity by optimizing the system, reducing primer-dimer rate.

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 2kb/min. It has 5' to 3' polymerase activity, but lacks of 3' to 5' exonuclease activity that results in 3'-dA overhangs PCR products. All components of the *HS*™ Mix are at optimal concentration for efficient amplification. It contributes to highly specific incorporation of primers and template.

Composition of the 2× HS™ Mix

0.3U/μl *HS*™ Taq DNA Polymerase, 2× *HS*™ PCR Buffer, 0.4mM dNTPs, 3.2mM $MgCl_2$, 0.02% bromophenol blue.

HS™ Mix buffer is a proprietary formulation optimized for robust performance in PCR.

Applications

- High throughput PCR
- High specificity PCR

- Routine PCR with high reproducibility
- Generation of PCR products for TA cloning

Features

- Convenient: only primers and template DNA are added when prepare final PCR
- High yields of PCR products with minimal optimization
- High efficiency: saving your time by simplifying the process
- Reproducible: lower contamination and pipetting error risk
- High sensitivity: compared to conventional Taq DNA Polymerase.

Basic PCR Protocol

All solutions should be thawed on ice, gently vortex and briefly centrifuge.

1. Add the following components to a sterile microcentrifuge tube sitting on ice or at room temperature:

Reagent	Quantity	Final concentration
2× *HS*™ Mix	25 µl	1×
Forward Primer	variable	0.4-1 µM
Reverse Primer	variable	0.4-1 µM
Template DNA	variable	10pg-1µg
Water, nuclease-free	to 50 µl	–

Recommendation amounts of template DNA in a 50 µl reaction mix:

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

2. Mix contents in the 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°C	3 minutes
25-35 Cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 1-10 minutes
Final Extension	72°C	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 nucleic acid dye staining. Use appropriate molecular weight standards.

Notes on cycling conditions

- *HS*™ Taq DNA Polymerase is suitable for high-specificity PCR applications.
- The half-life of *HS*™ Taq DNA Polymerase 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
- *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 reactions. Low amounts of starting template may require 40 cycles.

Guidelines for preventing contamination of PCR

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set-up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set-up.
- Always perform “no template control” (NTC) reactions to check for contamination.

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 25 µl *HS*™ Mix (2×) 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 25 µl *HS*™ Mix (2×) 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 25 µl *HS*™ Mix (2×) with 1µg E.coli [3H]-RNA (40000cpm/µg) for 4 hours at 37°C and 70°C.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.