HotStart Mix (2x)

#Cat: NB-03-0108 Size: 5x 1ml

Contents

NB-03-0108 (200 reactions of 50 µl each)	
2×PCR Plus Mix	5x 1 ml
Nuclease-free Water	5x 1 ml

Description

HotStart™ 2X Mix is a premixed, ready-to-use solution containing HotStart™ Taq DNA Polymerase, dNTPs, Mg2+ and Reaction Buffer at optimal concentrations for efficient amplification of DNA templates by PCR. To prepare the final PCR, only primers and template DNA are added. HotStart™ Taq 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. The mix has dramatic increased the sensitivity by adding enhancer.

HotStart™ Taq DNA Polymerase is a thermostable recombinant DNA polymerase derived from thermophilic bacterium Thermus aquaticus .Its molecular weight is 94 kDa. HotStart™ 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 components of the HotStart™ Mix are at optimal concentration for efficient amplification, which contributes to highly specific incorporation of primer and template.

Features

- Convenient –HS™ Taq DNA Polymerase in a ready-to-use Mix.
- High yields of PCR products with minimal optimization.
- Fast -saves time due to reduced number of pipetting steps.
- Reproducible -lower contamination and pipetting error risk.
- Higher sensitivity compared to conventional Taq DNA polymerase.

Applications

• High throughput PCR.

• High Specificity PCR

• Routine PCR with high reproducibility

• Generation of PCR products for TA cloning

Composition of the HotStart™ Mix

HSTaq DNA polymerase is supplied in 2X Taq buffer, dNTPs, 3 mM MgSO4 and bromophenol blue. HSTM mix buffer is a proprietary formulation optimized for robustperformance in PCR.

Store at -20°C

Repeated freeze-thaw cycles do not reduce the activity of the reactions.

Protocol for PCR

All solutions should be thawed on ice, gently vortexed and briefly centrifuged.

1. Add in a thin walled PCR tube on ice:

For a total 50µl reaction volume

Components	Volume	Final Concentration
2x HotStart™ Mix	25 µl	1 x
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 50µl 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. Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of thetube.

3. Overlay the sample with mineral oil or add an appropriate amount of wax. This step may beomitted if the thermal cycler is equipped with a heated lid.

4. Perform PCR using the following thermal cycling conditions:

Initial Denaturation	94°C	3 minutes
25-35 cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 1 min
Final extension	72°C	10 minutes

5. Maintain the reaction at 4°C. The samples can be stored at -20°C until use.

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

Guidelines for preventing contamination of PCR reaction

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 pipettetips 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*TM Mix (2X) with 1 µg of pBR322 DNA in 50 µl for 4 hours at 37°C and at 70°C.

Exodeoxyribonuclease Assay No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 25 µl of *HS*TM Mix (2X) with 1 µg of digested DNA in 50 µl for 4 hours at 37°C and at 70°C.

Ribonuclease Assay 0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25 µl of *HS*TM Mix (2X) with 1 µg of E.coli [3H]-RNA (40000cpm/µg) in 50 µl for 4 hours at 37°C.