HotStar PCR SuperMix

Cat. No.: SM201-0100 Size: 100 Reactions
Cat. No.: SM201-0002 Size: 2 Reactions
Store at -20°C (in a non-frost-free freezer)
Shipping Condition: Approved for shipment on wet or dry Ice

Description

HotStar PCR SuperMix provides qualified reagents for the amplification of nucleic acid templates by polymerase chain reaction (PCR). The mixture contains anti-*Taq* DNA polymerase antibody, Mg^{++}, dNTPs, and recombinant *Taq* DNA polymerase at concentrations sufficient to allow amplification during PCR. HotStar PCR SuperMix is supplied at 2X concentration to allow approximately 50% of the final reaction volume to be used for the addition of primer and template solutions. Reagents sufficient for 100 amplification reactions of 50 µl each are provided. Anti-*Taq* DNA polymerase antibody inhibits polymerase activity providing an automatic hot start and permits ambient temperature setup. Antibody-mediated hot starts improve PCR specificity and yield. Due to specific binding of the antibody, HotStar PCR SuperMix is present in an inactive form and is reactivated after a denaturation step in PCR cycling at 94°C. HotStar PCR SuperMix may be stored at either -20°C or 4°C. Storage at 4°C avoids the necessity of thawing the mix before assembling the PCR. Repeated freeze-thaw cycles might reduce performance or activity.

Kit contents

Component	size
HotStar PCR SuperMix	2 × 1.25 ml

This product is distributed for laboratory research only.
CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

Quality Control

HotStar PCR SuperMix is evaluated in a DNA polymerization activity assay that measures the percent of *Taq* DNA polymerase inhibition versus an uninhibited control. A functional assay is also performed. Components of HotStar PCR SuperMix are tested for the absence of DNase, RNase and exonuclease activities. Recombinant *Taq* DNA polymerase is tested for the absence of exonuclease, and double- and single-stranded endonuclease activities.

Guidelines and Recommendations

Since PCR is a powerful technique capable of amplifying trace amounts of DNA, all appropriate precautions should be taken to avoid cross contamination. Ideally, amplification reactions should be assembled in a DNA-free environment. Use of aerosol-resistant barrier tips is recommended. Take care to avoid contamination of HotStar PCR SuperMix with the primers or template DNA used in individual reactions. PCR products should be analyzed in an area separate from the reaction assembly area.
A standard 50 µl reaction uses 25 µl of HotStar PCR SuperMix and 25 µl of primer and template solutions. For the primer sets used in the development of HotStar PCR SuperMix, no decrease in product yield was observed if the amount of template and primer solution added is between a fraction of a microliter and 25 µl. Lower yield occurs as the Mg^{++} concentration drops to a suboptimal level. If the final Mg^{++} concentration is adjusted to 1.5 mM, the volume of primer and template solution that can be added to 25 µl of HotStar PCR SuperMix can exceed 50 µl.

Protocol

The following protocol is suggested as a starting point and guideline when using HotStar PCR SuperMix. We recommend assembling reactions on ice from pre-chilled components. This protocol is for a reaction size of approximately 50 µl. The reaction size may be adjusted as desired.
Note: For multiple reactions with common components, prepare a master mix of the components common to all reactions to reduce pipetting errors.
1. Set up reaction tubes/plates on ice.
2. Add the following components in any order to each reaction vessel.

	Volume (µl)
HotStar PCR SuperMix	25
Forward primer (10 µM)	1
Reverse primer (10 µM)	1
Template DNA solution	Variable
Add ddH_2O to	50

Note:
- Primers (200 nM final concentration per primer is recommended)
- Total volume of primer and template solution can be 0.5～25 µl.

3. Cap reaction vessels and load in thermal cycler at 94°C.
4. Perform of PCR amplification as follows:

Initial denature	94°C for 30 s～2 min	
Denature	94°C for 15～30 s	25～35 cycles
Anneal	55°C for 15～30 s	
Extend	72°C for 1 min per kb	

Related Ordering Information

Cat. No.	Description	Package
BK001	BLooK LED transilluminator	1 each
SL001-1000	Novel Juice	1 ml
SL003-1000	Novel Green Plus	500 µl (20,000X in DMSO)
SM200-0100	PCR SuperMix	100 Reactions
SM203-0100	OnePCR™	100 Reactions
SM205-0100	OnePCR™ HiFi	100 Reactions
SM206-0100	OnePCR™ Star	100 Reactions
SM300-0050	One-Step RT-PCR System	50 Reactions
SM303-0050	GScript RTase	50 Reactions
SM601-0100	GDP Hifi DNA Polymerase	100 U
SD101-0100	OneMARK 100	600 µl
SD110-0100	OneMARK B	600 µl

Caution

- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- All products are for research use only.