

Products	Cat #	Pack Size
BioThermStar Hot-Start PCR Mix™ (100 rx/25ul)	GC-041-1000	1 ml

DESCRIPTION

BioThermStar PCR Mix™ is a 2.5x concentrated reagent mix for **Hot Start PCR** comprising BioThermStar™ DNA Polymerase, 2.5x PCR-Buffer with 3.75 mM MgCl₂, 500µM of each dNTP and stabilizers. The 2.5x concentration of the Mix provides high versatility of setting-up **Hot Start PCR reactions**. Up to 60% of the final reaction volume can be used for the addition of primer and template DNA solutions, co-solvents or other additives, if necessary.

APPLICATION

BioThermStarMix™ is suitable and tested for amplification of genomic targets ranging from 100 bp to 4 kb and of episomal targets (lambda phage; plasmids) up to 10 kb under various reaction conditions.

- **high through-put Hot Start PCR**
- routine diagnostic Hot Start **PCR requiring high reproducibility**
- DNA sequencing template preparation

STORAGE CONDITION

BioThermStarMix™ can be stored at either -20°C in a freezer or at 2-8°C in a usual refrigerator. Shipment at ambient temperatures is possible without reduction of Hot Start PCR performance and activity. Storage at 2-8°C is convenient for easy and time-saving assembling of the Hot Start PCR assays. Storage of BioThermStarMix™ at -20°C is recommended for long-term storage after the mix has been used once under non-sterile conditions. Multiple freezing and thawing do not affect the performance or activity of the mix. At 2-8°C the BioThermStarMix™ is at least stable for 12 months, in frozen state for 2 years.

NOTE

Do not contaminate the BioThermStarMix™ with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

It is very important to mix the BioThermStarMix™ before use to avoid localized concentration!

PROTOCOL

1. Place the Hot Start tubes on ice.
2. Prepare first a template/primer mix according to the volumes given in the table below for different reaction volumes. Mix the template/primer mix and chill on ice.
3. Dispense now the corresponding volume of BioThermStarMix™ (20 µl for a 50 µl reaction) followed by the template/primer mix into each reaction tube. Close tubes and mix well. Spin down shortly, if necessary, and chill the tubes on ice.
4. Start the Hot Start program. Transfer the tubes directly from the ice into the thermal cycler when the temperature of the block has reached 95°C. **Incubate cca. 7 min. at 95°C before cycling.**

IMPORTANT NOTE

The stabilizers in the BioThermStarMix™ are potential growth substrates of bacterial contaminations! If the Mix has been opened and used under non-sterile conditions, the residual moiety should be used

during the next 2 weeks with intermediate storage at 2-8°C or should be frozen at -20°C for longer storage!

REACTION VOLUMES

PCR Volume	Sterile redi-stilled H ₂ O	Sense Primer	Antisense Primer	Template DNA	2.5x PCR Mix
100 µl	up to 60 µl	x µl	y µl	z µl	40 µl
50 µl	up to 30 µl	x µl	y µl	z µl	20 µl
25 µl	up to 15 µl	x µl	y µl	z µl	10 µl
20 µl	up to 12 µl	x µl	y µl	z µl	8 µl
Final concentrations:		200 nM	200 nM	1-100 ng	1x

Other variable reaction conditions (temperatures, cycling times, concentrations of template, primers, magnesium and polymerase) have to be optimized empirically for each template/primers combination. Most PCR applications work at the standard concentration of 1,5 mM Mg²⁺ provided with 1x diluted PCR Mix. Optimal Mg²⁺ concentration higher than 1,5 mM

can be adjusted using a separate magnesium solution or by increasing stepwise (5 µl increments) the amount of BioThermStarMix™ added to the reaction assay. This approach can also be used to improve the product yield in amplification of difficult targets on complex template DNA (see table below).

Optimization by adding variable amounts of concentrated BioThermStarMix™ to a 50 µl-assay:

Volume of 2,5x PCR Mix	Volume of template/primer mix	Final Mg ²⁺ Concentration	Final dNTP-Concentration	Taq units per reaction	Final concentrations
20 µl	30 µl	1.5 mM	200 µM	1.25 u	1x
25 µl	25 µl	2.0 mM	250 µM	1.6 u	1.25x
30 µl	20 µl	2.25 mM	300 µM	1.9 u	1.5x