2X Hot Start PCR Master Mix

Cód.: 13-10502-01 **100 reactions**

Stored at –20°C.

2X Hot Start PCR Master Mix is also stable for two months at 4°C, so for frequent use, an aliquot may be kept at 4°C.

Description

2X Hot Start PCR Master Mix is an optimized ready-to-use solution containing Taq DNA Polymerase, anti-Taq DNA polymerase antibody, PCR reaction buffer, $MgCl_2$, dNTPs and stabilizers. It is ideally suited to routine PCR applications from templates including pure DNA solutions, bacterial colonies and cDNA products. It can amplify DNA fragments up to 5 kb.

Anti-Taq DNA polymerase antibody inhibits polymerase activity providing an automatic "hot start" and allows convenient setup of PCR reactions at ambient temperature. Due to specific binding of the antibody, 2X Hot Start PCR Master Mix is present in an inactive form and is reactivated after a denaturation step in PCR cycling at 95°C.

Antibody-mediated hot start can also improve the specificity of PCR and yield of amplified DNA fragments.

Application

- PCR
- Primer Extension
- Microarray Analysis
- High-Throughput PCR

List of components

Each product contains sufficient reagents to perform 50 µL PCR reactions.

Cat. No.	13-10502-01	100 reactions
2 x 1.25 mL	2X Hot Start PCR Master Mix	TRANSPARENT CAP

Protocol

This protocol is for a reaction size of 50 µL. The reaction size may be adjusted as desired.

For multiple reaction, prepare a master mix of the components common to all reactions to reduce pipetting errors.

1. Thaw the PCR Master Mix at room temperature. When thawed, resuspend the Master Mix by vortexing and then briefly centrifuge to collect the solution in the bottom of the tube.

2. Prepare the following reaction mix for each sample:

Component	Volume	Final Conc.
2X Hot Start PCR Master Mix	25 µL	1X
10 µM Forward Primer	1 µL	0.2 µM (0.05–1 µM)
10 µM Reverse Primer	1 µL	0.2 µM (0.05–1 µM)
DNA template	variable	< 1 µg
Nuclease-free water	to 50 µL	

Recommended amplification parameters for PCR:

Stage	Step	Temp	Time
Hold	Initial denaturation	95°C	2 min
Cycles (25 to 45 cycles)	Denature	95°C	30 sec
	Anneal	55°C (Primer T_m)	30 sec
	Extend	72°C	60 sec per kb
Hold	Final extension	72°C	5 min

General Guidelines

DNA Template:

Use of high quality, purified DNA templates greatly enhances the success of amplification. Recommended amounts of DNA template for a 50 µl reaction are as follows:

Genomic DNA 1 ng–1 µg

Plasmid or Viral DNA 1 pg–1 ng

Primers:

Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as Primer3 (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) can be used to design or analyze primers. The final concentration of each primer in a reaction may be 0.05–1 µM, typically 0.1–0.5 µM.

Mg++ and additives:

Mg++ concentration of 1.5–2.0 mM is optimal for most PCR products generated with Taq DNA Polymerase. The final Mg++ concentration in 1X Hot Start PCR Master Mix is 1.5 mM. This supports satisfactory amplification of most amplicons. However, Mg++ can be further optimized in 0.5 or 1.0 mM increments using $MgCl_2$.

Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO, betaine or formamide. See additional information about PCR Additives at the following link:

http://www.staff.uni-mainz.de/lieb/additiva.html

Denaturation:

An initial denaturation of 30 seconds at 95°C is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 2–5 minutes at 95°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5 minutes denaturation at 95°C is recommended.

A denaturation step of 15–30 seconds at 95°C is recommended during the PCR cycles.

Annealing:

The annealing step is typically 15–60 seconds. Annealing temperature is based on the T_m of the primer pair and is typically 45–68°C. Annealing temperatures can be optimized by doing a temperature gradient PCR starting 5°C below the calculated T_m.

Extension:

The extension reaction is typically performed at the optimal temperature for Taq DNA polymerase: 72°C. Allow 1 minute for every 1kb of DNA to be amplified. A final extension of 5 minutes at 72°C is recommended.

Cycle number:

Generally, 25–35 cycles yields sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.

PCR product:

The PCR products generated using Taq DNA Polymerase contain dA overhangs at the 3´–end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

Quality Control Assays

Functional Assay:

2X Hot Start PCR Master Mix is tested for performance in the polymerase chain reaction (PCR) to amplify a 2kb region of the beta-globin gene from 50 ng of human genomic DNA. The resulting PCR product is visualized on an ethidium bromide-stained agarose gel.

Amplification parameters for PCR of beta-globin gene:

Stage	Step	Temp	Time
Hold	Initial denaturation	95°C	2 min
30 cycles	Denature	95°C	30 sec
	Anneal	55°C	15 sec
	Extend	72°C	60 sec
Hold	Final extension	72°C	5 min

Primers used for amplification of human beta-globin gene:

2kb_Forward (5' - TCT TGG CAG AGT GTA TGT GTC - 3')

2kb_ Reverse (5' - TAA CCG ATG AGA TCA ACT GGA A - 3')

Nuclease Assays:

No contaminating endonuclease or exonuclease activity detected.