Hot Start *Taq* 2X Master Mix

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M0496S 0111602180121

M0496S

100 reactions (50 µl vol) **Lot: 0111602**

RECOMBINANT Store at –20°C Exp: 2/18

Description: Hot Start *Taq* 2X Master Mix is an optimized ready-to-use solution containing Hot Start *Taq* DNA Polymerase, dNTPs, $MgCl_2$, KCl, and stabilizers. It is ideally suited to routine PCR applications from templates including pure DNA solutions, bacterial colonies, and cDNA products. It is recommended for amplification up to 5 kb.

Hot Start *Taq* DNA Polymerase is a mixture of *Taq* DNA Polymerase and an aptamer-based inhibitor. The inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal cycling conditions allowing reactions to be set up at room temperature. The aptamer-based hot start does not require a separate high temperature incubation step to activate the enzyme. Hot Start *Taq* DNA Polymerase possesses a 5´→ 3´ polymerase activity (1,2,3) and a 5´ flap endonuclease activity (4,5).

Source: An *E. coli* strain that carries the *Taq* DNA Polymerase gene from *Thermus aquaticus* YT-1

Application:
- High-specificity PCR
- Routine PCR
- High-throughput PCR
- Microarray Analysis
- Colony PCR

Reaction Conditions: 1X Hot Start *Taq* Master Mix, DNA template and primers in a total reaction volume of 25 or 50 µl.

Hot Start *Taq* 1X Master Mix:
10 mM Tris-HCl (pH 8.6 @ 25°C)
50 mM KCl
1.5 mM $MgCl_2$
25 units/ml Hot Start *Taq* DNA polymerase
0.2 mM dNTPs each
5% Glycerol

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 15 nmol of dNTP into acid insoluble material in 30 minutes at 75°C.

Unit Assay Conditions: 1X ThermoPol® Reaction Buffer, 200 µM dNTPs including [^{3}H]-dTTP and 15 nM primed M13 DNA.

Heat Inactivation: No

Quality Control Assays

High-Sensitivity PCR Assay: 30 cycles of PCR amplification of 20 pg Lambda DNA in a 50 µl reaction containing 100 ng human genomic DNA, 0.2 µM primers and 1X Hot Start *Taq* Master Mix result in a single 2 kb Lambda product. In non-Hot Start PCR control reactions, characteristic non-specific bands are produced from this set of primers and template.

3´→ 5´ Exonuclease Activity: Incubation of a 20 µl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of *Taq* DNA Polymerase with 10 nM fluorescent internally-labeled oligonucleotide for 30 minutes at either 37°C or 75°C yields no detectable 3´→ 5´ degradation as determined by capillary electrophoresis.

Endonuclease Activity: Incubation of a 50 µl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of *Taq* DNA Polymerase with 1 µg of supercoiled ϕX174 DNA for 4 hours at 75°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Inhibition Assay: Greater than 95% inhibition is observed after a 16 hour incubation at 25°C in a 50 µl primer extension assay containing 2.5 units of Hot Start *Taq* DNA Polymerase in 1X ThermoPol Reaction Buffer with 200 µM dNTPs including [^{3}H]-dTTP and 15 nM primed single-stranded M13mp18

PCR

The following guidelines are provided to ensure successful PCR using New England Biolabs' Hot Start *Taq* 2X Master Mix. These guidelines cover routine PCR. Amplification of templates with high GC content, high secondary structure, low template concentrations, or amplicons greater than 5 kb may require further optimization.

Reaction setup:

Due to the hot start nature of the enzyme, reactions can be assembled on the bench at room temperature and transferred to a thermocycler. *No separate activation step is required to release the inhibitor from the enzyme.*

Add to a sterile thin-walled PCR tube:

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
Template DNA	variable	variable	<1,000 ng
Hot Start *Taq* 2X Master Mix	12.5 µl	25 µl	1X
Nuclease-Free Water	to 25 µl	to 50 µl	

Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary.

Transfer PCR tubes to a PCR machine and begin thermocycling:

Thermocycling conditions for a routine PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C 45–68°C 68°C	15–30 seconds 15–60 seconds 1 minute/kb
Final Extension	68°C	5 minutes
Hold	4–10°C	

General Guidelines:

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

DNA	AMOUNT
Genomic	1 ng–1 µg
Plasmid or Viral	1 pg–1 ng

2. Primers:
Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as PrimerSelect™ and Primer 3 (http://frodo.wi.mit.edu/primer3) can be used to design or analyze primers. The final concentration of each primer in a PCR may be 0.05–1 µM, typically 0.1–0.5 µM.

3. Mg^{++} and additives:
Mg^{++} concentration of 1.5–2.0 mM is optimal for most PCR products generated with Hot Start *Taq* DNA Polymerase. The final Mg^{++} concentration in 1X Hot Start *Taq* 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 $MgCl_2$ (sold separately).
Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO (6) or formamide (7).

4. Denaturation:
No separate activation step is required to release the hot start inhibitor from the enzyme. 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 2–4 minutes at 95°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5 minute denaturation at 95°C is recommended.
During thermocycling a 15–30 second denaturation at 95°C is recommended.

5. 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. We recommend using NEB's T_m Calculator, available at www.neb.com/TmCalculator to determine appropriate annealing temperatures for PCR.
When primers with annealing temperatures above 68°C are used, a 2-step PCR protocol is possible (see #8).

6. Extension:
The recommended extension temperature is 68°C. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 68°C is recommended.

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

(see other side)

8. 2-step PCR:
   When primers with annealing temperatures above 68°C are used, a 2-step thermocycling protocol is possible.

   Thermocycling conditions for a routine 2-step PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C	15–30 seconds
	68°C	1 minute/kb
Final Extension	68°C	5 minutes
Hold	4–10°C	

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

Notes: Hot Start *Taq* 2X Master Mix is stable for fifteen freeze-thaw cycles when stored at –20°C.

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

References:

1. Chien, A., Edgar, D.B. and Trela, J.M. (1976) *J. Bact.,* 127, 1550–1557.
2. Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I. (1980) *Biokhimiya,* 45, 644-651.
3. Lawyer, F.C. et al. (1993) *PCR Methods and Appl.,* 2, 275–287.
4. Longley, M.J., Bennett, S.E. and Mosbaugh D.W. (1990) *Nucleic Acids Res.,* 18, 7317–7322.
5. Lyamichev, V., Brow, M.A. and Dahlberg, J.E. (1993) *Science,* 260, 778–783.
6. Sun, Y., Hegamyer, G. and Colburn, N. (1993) *Biotechniques,* 15, 372–374.
7. Sarkar, G., Kapelner, S. and Sommer, S.S. (1990) *Nucleic Acids Res.,* 18, 7465.

Companion Products Sold Separately:

Hot Start *Taq* DNA Polymerase
#M0495S 400 units
#M0495L 1,000 units

Magnesium Chloride ($MgCl_2$) Solution
#B9021S 6.0 ml