

# Hot Start Taq 2X Master Mix



1-800-632-7799  
info@neb.com  
www.neb.com



M0496S 009150917091

## M0496S



100 reactions (50 µl vol)

Lot: 0091509

RECOMBINANT Store at -20°C Exp: 9/17

**Description:** Hot Start *Taq* 2X Master Mix is an optimized ready-to-use solution containing Hot Start *Taq* DNA Polymerase, dNTPs, MgCl<sub>2</sub>, 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<sub>2</sub>  
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 [<sup>3</sup>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 [<sup>3</sup>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 <i>Taq</i> 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	15–30 seconds
	45–68°C	15–60 seconds
	68°C	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<sup>++</sup> and additives:**  
Mg<sup>++</sup> concentration of 1.5–2.0 mM is optimal for most PCR products generated with Hot Start *Taq* DNA Polymerase. The final Mg<sup>++</sup> concentration in 1X Hot Start *Taq* Master Mix is 1.5 mM. This supports satisfactory amplification of most amplicons. However, Mg<sup>++</sup> can be further optimized in 0.5 or 1.0 mM increments using MgCl<sub>2</sub> (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<sub>m</sub> 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<sub>m</sub>. We recommend using NEB's T<sub>m</sub> Calculator, available at [www.neb.com/TmCalculator](http://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:**

- Chien, A., Edgar, D.B. and Trela, J.M. (1976) *J. Bact.*, 127, 1550–1557.
- Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I. (1980) *Biokhimiya*, 45, 644-651.
- Lawyer, F.C. et al. (1993) *PCR Methods and Appl.*, 2, 275–287.
- Longley, M.J., Bennett, S.E. and Mosbaugh D.W. (1990) *Nucleic Acids Res.*, 18, 7317–7322.
- Lyamichev, V., Brow, M.A. and Dahlberg, J.E. (1993) *Science*, 260, 778–783.
- Sun, Y., Hegamyer, G. and Colburn, N. (1993) *Biotechniques*, 15, 372–374.
- 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<sub>2</sub>) Solution  
#B9021S 6.0 ml



NEW ENGLAND BIOLABS® and THERMOPOL® are registered trademarks of New England Biolabs, Inc.

PRIMERSELECT™ is a trademark of DNASTar, Inc., Madison, Wisconsin, USA.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Notice to Purchaser: Nucleic acid-based aptamers for use with thermophilic DNA polymerases are licensed exclusively by New England Biolabs, Inc. from SomaLogic, Inc. (See Patent Nos. 5,475,096; 5,670,637; 5,696,249; 5,874,557; and 5,693,502). New England Biolabs, Inc. gives the Buyer/User a non-exclusive license to use the aptamer-based Hot-Start *Taq* 2X Master Mix for RESEARCH PURPOSES ONLY. Commercial use of the aptamer-based Hot Start *Taq* 2X Master Mix requires a license from New England Biolabs, Inc. Please contact busdev@neb.com for more information.

Certain applications in which this product can be used may be covered by third party patents or other intellectual property rights. It is the sole responsibility of the Buyers/Users of this product to determine whether they may be required to obtain any third party intellectual property rights (which may include, without limitation, a license to perform any patented application) depending upon the particular application in which the product is used.