# Hot Start *Taq* 2X Master Mix

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M0496S 002121214121

# M0496S

**100 reactions (50 µl vol)** **Lot: 0021212**
**RECOMBINANT Store at –20°C** **Exp: 12/14**

**Description:** Hot Start *Taq* 2X Master Mix is an optimized ready-to-use solution containing Hot Start *Taq* DNA Polymerase, dNTPs, Standard *Taq* Reaction Buffer, 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 200 µg/ml activated Calf Thymus 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 Hot Start *Taq* 1X 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 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. $Mg^{++}$ 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 Hot Start *Taq* 1X 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:**


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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


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