One*Taq*® Hot Start Quick-Load® 2X Master Mix with Standard Buffer

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M0488S 018150316031

M0488S

100 reactions (50 µl vol) **Lot: 0181503**

RECOMBINANT Store at –20°C Exp: 3/16

Description: One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer is an optimized, ready-to-use blend of *Taq* and Deep Vent$_R$™ DNA Polymerases combined with an aptamer-based inhibitor. This enzyme blend is ideally suited to routine PCR applications from templates, including pure DNA solutions, bacterial colonies and cDNA products. The 3´→5´ exonuclease activity of Deep Vent DNA Polymerase increases the fidelity and robust amplification of *Taq* DNA Polymerase (1). The hot start nature of the enzyme offers convenience with decreased interference from primer dimers and secondary products. The convenient Quick-Load master mix formulation contains dNTPs, $MgCl_2$, buffer components and stabilizers as well as two commonly used tracking dyes for DNA gels. On a 1% agarose gel in 1X TBE, Xylene Cyanol FF migrates at ~4 kb and Tartrazine migrates at ~10 bp. Both dyes are present in concentrations that do not mask co-migrating DNA bands.

Source: An *E. coli* strain that carries the *Taq* DNA Polymerase gene from *Thermus aquaticus* YT-1 and an *E. coli* strain that carries the Deep Vent$_R$ DNA Polymerase gene from *Pyrococcus* species GB-D.

Applications:

- High Sensitivity PCR
- High Throughput PCR
- Routine PCR
- AT-rich PCR
- Colony PCR
- Long PCR (up to ~6 kb genomic)

Reaction Conditions: 1X One*Taq* Hot Start Quick-Load Master Mix with Standard Buffer, DNA template and primers in a total reaction volume of 50 µl.

1X One*Taq* Hot Start Quick-Load Master Mix with Standard Buffer:
20 mM Tris-HCl (pH 8.9 @ 25°C)
1.8 mM $MgCl_2$
22 mM NH_4Cl
22 mM KCl
0.2 mM dNTPs
5% glycerol
0.06% IGEPAL® CA-630
0.05% Tween® 20
Xylene Cyanol FF
Tartrazine
25 units/ml One*Taq* Hot Start DNA Polymerase

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.

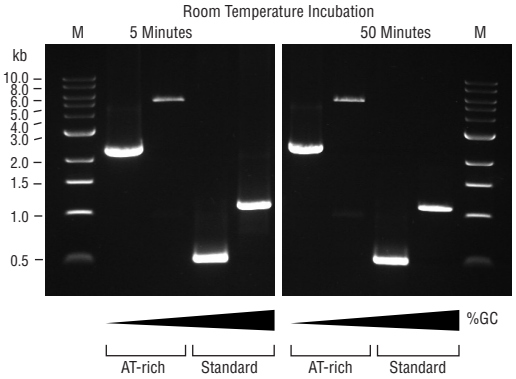
Unit Assay Conditions: 1X ThermoPol® Reaction Buffer, 200 µM dNTPs including [^{3}H]-dTTP and 15 nM primed M13 DNA.

Heat Inactivation: No

Quality Control Assays

5 kb Lambda PCR: 25 cycles of PCR amplification of 5 ng Lambda DNA with 1X One*Taq* Hot Start Quick-Load Master Mix with Standard Buffer in a 25 µl reaction in the presence of 0.2 µM primers resulted in the expected 5 kb product.

Note: Product specifications for individual components in the One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer are available separately.



Amplification of a selection of sequences with varying GC content from human and C. elegans genomic DNA using OneTaq Hot Start DNA Polymerase. GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).

PCR

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (2). *Taq* DNA Polymerase is an enzyme widely used in PCR (3). The following guidelines are provided to ensure successful PCR using New England Biolabs' One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer. These guidelines cover routine PCR reactions. Amplification of templates with high GC content, high secondary structure or with low template concentrations may require further optimization.

Reaction Setup:
Due to the presence of the inhibitor, 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.*

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer	12.5 µl	25 µl	1X
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM
Template DNA	variable	variable	<1,000 ng
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. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Transfer PCR tubes to a PCR machine and begin thermocycling:

Thermocycling Conditions for a Routine PCR:

STEP	TEMP	TIME
Initial Denaturation	94°C	30 seconds
30 Cycles	94°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 reactions. 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 Primer3 (http://frodo.wi.mit.edu/primer3) can be used to design or analyze primers. The final concentration of each primer in a PCR reaction may be 0.05–1 µM, typically 0.2 µM.

3. Mg^{++} and additives:
Mg^{++} concentration of 1.5–2.0 mM is optimal for most PCR products generated with One*Taq* DNA Polymerase. The final Mg^{++} concentration in 1X One*Taq* Hot Start Quick-Load Master Mix with Standard Buffer is 1.8 mM. This supports satisfactory amplification of most amplicons. However, Mg^{++} can be further optimized in 0.2 mM increments using $MgCl_2$ (sold separately).

   For amplification of difficult targets, like GC-rich sequences, we recommend One*Taq* Hot Start Quick-Load 2X Master Mix with GC Buffer (sold separately). Alternatively, DMSO (4) or formamide (5) may be used.

4. Denaturation:
An initial denaturation of 30 seconds at 94°C is sufficient to amplify most targets from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 2–4 minutes at 94°C is recommended prior to PCR cycling to fully denature the template. Alternatively, use One*Taq* Hot Start 2X Master Mix with GC Buffer. With colony PCR, an initial 2–5 minute denaturation at 94°C is recommended to lyse cells.

   During thermocycling a 15–30 second denaturation at 94°C is recommended.

(see other side)

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.

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 yields sufficient product. Up to 45 cycles may be required to detect low copy number targets.

8. 2-step PCR:
   When primers with annealing temperatures of 68°C or above are used, a 2-step thermocycling protocol (combining annealing and extension into one step) is possible.

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

Notes:

One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer is stable for fifteen freeze-thaw cycles when stored at –20°C

One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer is also stable for one month at 4°C, so for frequent use, an aliquot may be kept at 4°C.

References:

1. Barnes, W.M. (1994) *Proc. Natl. Acad. Sci. USA,* 91, 2216–2220.
2. Saiki R.K. et al. (1985) *Science,* 230, 1350–1354.
3. Powell, L.M. et al. (1987) *Cell,* 50, 831–840.
4. Sun, Y., Hegamyer, G. and Colburn, N. (1993) *Biotechniques*, 15, 372–374.
5. Sarkar, G., Kapelner, S. and Sommer, S.S. (1990) *Nucleic Acids Res.*, 18, 7465.

Companion Products Sold Separately:

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

One*Taq* Hot Start Quick-Load 2X Master Mix with GC Buffer
#M0489S 100 Reactions
#M0489L 500 Reactions

One*Taq* Hot Start 2X Master Mix with Standard Buffer
#M0484S 100 Reactions
#M0484L 500 Reactions

One*Taq* Hot Start 2X Master Mix with GC Buffer
#M0485S 100 Reactions
#M0485L 500 Reactions

One*Taq* DNA Polymerase
#M0480S 200 units
#M0480L 1,000 units
#M0480X 5,000 units

One*Taq* Hot Start DNA Polymerase
#M0481S 200 units
#M0481L 1,000 units
#M0481X 5,000 units