OneTaq® Hot Start Quick-Load® 2X Master Mix with GC Buffer

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M0489S 011130314031

M0489S

100 reactions (50 µl vol) **Lot: 0111303**

RECOMBINANT Store at –20°C Exp: 3/14

Description: One*Taq* Hot Start Quick-Load 2X Master Mix with GC 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 PCR applications from GC-rich 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 master mix formulation contains dNTPs, $MgSO_4$, 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:

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

Reagents Supplied with Enzyme:
One*Taq* High GC Enhancer

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

1X One Taq **Hot Start Quick-Load Master Mix with GC Buffer:**
80 mM Tris-SO_4 (pH 9.2 @ 25°C)
2 mM $MgSO_4$
20 mM $(NH_4)_2SO_4$
0.2 mM dNTPs
5% Glycerol
5% DMSO
0.06% IGEPAL® CA-630
0.05% Tween® 20
Xylene Cyanol FF
Tartrazine
25 units/ml One*Taq* Hot Start DNA Polymerase

OneTaq **High GC Enhancer:**
10 mM Tris-HCl (pH 9.2 @ 25°C)
25% DMSO
25% 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.

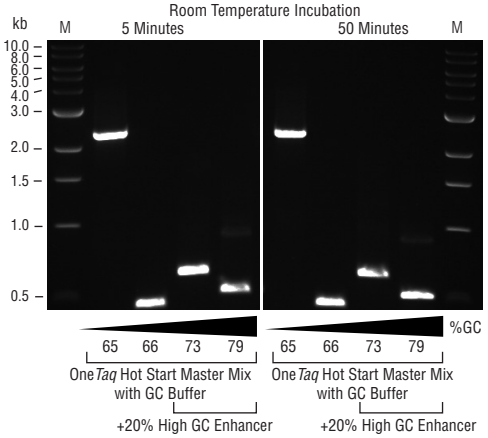
Heat Inactivation: No

Quality Control Assays

Buffer-dependent GC-rich (> 65% GC) PCR:
30 cycles of PCR amplification of 10 ng of human genomic DNA with 1X One*Taq* Hot Start Quick-Load Master Mix with GC Buffer in a 25 µl reaction in the presence of 0.2 µM primers resulted in the buffer-dependent production of the 737 bp GC-Rich product.

Enhancer-dependent High GC (> 70% GC) PCR:
30 cycles of PCR amplification of 10 ng of human genomic DNA with 1X One*Taq* Hot Start Quick-Load Master Mix with GC Buffer in a 25 µl reaction in the presence 0.2 µM primers and 20% One*Taq* High GC Enhancer resulted in the enhancer-dependent production of the 627 bp high GC product.

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



Amplification of a selection of sequences with varying GC content from human genomic DNA using One*Taq* 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 GC Buffer. These guidelines cover routine PCR reactions. Specialized applications 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 GC Buffer	12.5 µl	25 µl	1X
(One*Taq* High GC Enhancer, optional)*	*(2.5–5 µl)*	*(5–10 µl)*	*(10–20%)*
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	

*For extremely difficult or high GC amplicons, the addition of 10–20% One*Taq* High GC Enhancer may improve amplification.

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 GC Buffer is 2 mM. This supports satisfactory amplification of most amplicons. However, Mg^{++} can be further optimized in 0.2 mM increments using $MgSO_4$ (sold separately).

   Amplification of extremely difficult targets may be improved by the addition of 10–20% One*Taq* High GC Enhancer (included).

(see other side)

4. Denaturation:
   No separate activation step is required to release the hot start inhibitor from the enzyme. An initial denaturation of 30 seconds at 94°C is sufficient to amplify most targets from pure DNA templates. For difficult templates, a longer denaturation of 2–4 minutes at 94°C is recommended prior to PCR cycling to fully denature the template. 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.

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* Hot Start 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 GC Buffer is stable for fifteen freeze-thaw cycles when stored at –20°C.

One*Taq* Hot Start Quick-Load 2X Master Mix with GC 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.

Companion Products Sold Separately:

Magnesium Sulfate ($MgSO_4$) Solution

#B1003S	6.0 ml

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

#M0488S	100 reactions
#M0488L	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