

**OneTaq® Hot Start
2X Master Mix with
GC Buffer**



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M0485S



100 reactions (50 µl vol) Lot: 0111409

RECOMBINANT Store at -20°C Exp: 3/16

Description: OneTaq Hot Start 2X Master Mix with GC Buffer is an optimized 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, MgSO₄ and other buffer components and stabilizers listed below, requiring only the addition of primers and DNA template for robust amplification.

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:

OneTaq High GC Enhancer

Reaction Conditions: 1X OneTaq Hot Start Master Mix with GC Buffer, DNA template and primers in a total reaction volume of 50 µl.

1X OneTaq Hot Start Master Mix with GC Buffer:

- 80 mM Tris-SO₄ (pH 9.2 @ 25°C)
- 2 mM MgSO₄
- 20 mM (NH₄)₂SO₄
- 0.2 mM dNTPs
- 5% Glycerol
- 5% DMSO
- 0.06% IGEPAL® CA-630
- 0.05% Tween® 20
- 25 units/ml OneTaq 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 [³H]-dTTP and 200 µg/ml activated Calf Thymus DNA.

Heat Inactivation: No

Quality Control Assays

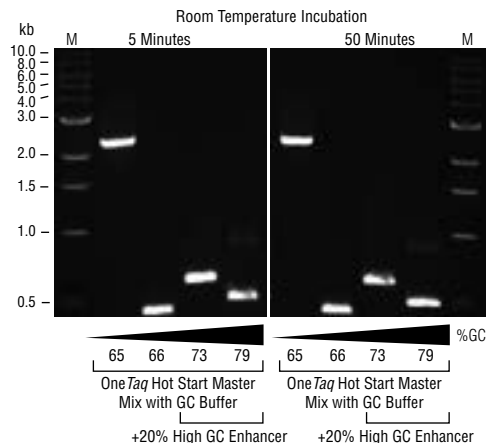
Buffer-dependent GC-rich (> 65% GC) PCR:

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

Note: Product specifications for individual components in the OneTaq Hot Start 2X Master Mix with GC Buffer are available separately.



Amplification of a selection of sequences with varying GC content from human genomic DNA using OneTaq Hot Start 2X Master Mix with GC Buffer. The presence or absence of an extended room temperature incubation does not affect performance. 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' OneTaq 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
OneTaq Hot Start 2X Master Mix with GC Buffer	12.5 µl	25 µl	1X
(OneTaq 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% OneTaq 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	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 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 OneTaq DNA Polymerase. The final Mg⁺⁺ concentration in 1X OneTaq Hot Start 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 MgSO₄ (sold separately).

Amplification of extremely difficult targets may be improved by the addition of 10–20% OneTaq 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 OneTaq DNA Polymerase contain dA overhangs at the 3' end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

Notes:

OneTaq Hot Start 2X Master Mix with GC Buffer is stable for fifteen freeze-thaw cycles when stored at –20°C.

OneTaq Hot Start 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:

- Barnes, W.M. (1994) *Proc. Natl. Acad. Sci. USA*, 91, 2216–2220.
- Saiki R.K. et al. (1985) *Science*, 230, 1350–1354.
- Powell, L.M. et al. (1987) *Cell*, 50, 831–840.

Companion Products Sold Separately:

Magnesium Sulfate ($MgSO_4$) Solution
#B1003S 6.0 ml

OneTaq Hot Start 2X Master Mix
with Standard Buffer
#M0484S 100 reactions
#M0484L 500 reactions

OneTaq Hot Start Quick-Load® 2X Master Mix with
Standard Buffer
#M0488S 100 reactions
#M0488L 500 reactions

OneTaq Hot Start Quick-Load 2X Master Mix with
GC Buffer
#M0489S 100 reactions
#M0489L 500 reactions

OneTaq DNA Polymerase
#M0480S 200 units
#M0480L 1,000 units
#M0480X 5,000 units

OneTaq Hot Start DNA Polymerase
#M0481S 200 units
#M0481L 1,000 units
#M0481X 5,000 units



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