Quick-Load® *Taq* 2X Master Mix

M0271S 023121213122

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M0271S

100 reactions (50 µl vol) Lot: 0231212

RECOMBINANT Store at –20°C Exp: 12/13

Description: Taq DNA Polymerase is a thermostable DNA polymerase that possesses a 5´ 3´ polymerase activity (1,2,3) and a 5´ flap endonuclease activity (4,5).

Quick-Load Taq 2X Master Mix is an optimized ready-to-use solution containing Taq DNA Polymerase, dNTPs, $MgCl_2$, KCl, tracking dyes and stabilizers. The presence of two commonly used tracking dyes for DNA gels, Orange G and Xylene Cyanol FF, gives the master mix a green color, allowing direct loading of the PCR reaction product onto agarose gels. On a 1% agarose gel in 1X TBE, Xylene Cyanol FF migrates at approximately 4 kb and Orange G migrates at approximately 50 bp. The amount of tracking dyes included does not mask co-migrating DNA bands. Quick-Load Taq 2X Master Mix is ideally suited to routine PCR applications from templates including pure DNA solutions, bacterial colonies, and cDNA products. It can amplify up to 4 kb from complex genomic DNA or up to 5 kb from lambda DNA.

Source: An E. coli strain that carries the Taq DNA Polymerase gene from Thermus aquaticus YT-1

Application:

- PCR
- Primer Extension
- High-Throughput PCR
- Colony PCR

Reagents Supplied with Enzyme:
25 mM $MgCl_2$

Reaction Conditions: 1X Quick-Load Taq Master Mix, DNA template and primers in a total reaction volume of 25 or 50 µl.

1X Quick-Load Taq Master Mix:
10 mM Tris-HCl (pH 8.6, @ 25°C)
50 mM KCl
1.5 mM $MgCl_2$
50 units/ml Taq DNA Polymerase
0.2 mM each dNTP
5% glycerol
0.08% IGEPAL® CA-630
0.05% Tween® 20
0.024% Orange G
0.0025% Xylene Cyanol FF

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10 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

5 kb Lambda PCR: 25 cycles of PCR amplification of 5 ng Lambda DNA, 0.2 µM primers and 1X Quick-Load Taq Master Mix results in the expected 5 kb product.

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.

PCR

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (6). Taq DNA Polymerase is an enzyme widely used in PCR (7). The following guidelines are provided to ensure successful PCR using New England Biolabs' Quick-Load Taq 2X Master Mix. These guidelines cover routine PCR reactions. 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:

We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (95°C).

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)
Quick-Load *Taq* 2X Master Mix	12.5 µl	25 µl	1X
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 from ice to a PCR machine with the block preheated to 95°C 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 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 0.1–0.5 µM.

3. Mg^{++} and additives:
   Mg^{++} concentration of 1.5–2.0 mM is optimal for most PCR products generated with Taq DNA Polymerase. The final Mg^{++} concentration in 1X Quick-Load Taq 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 $MgCl_2$.

   Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO (8) or formamide (9).

4. Denaturation:
   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 60°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 yields 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 60°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
	60–68°C	1 minute/kb
Final Extension	60–68°C	5 minutes
Hold	4–10°C	

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

Notes: Quick-Load Taq 2X Master Mix is stable for fifteen freeze-thaw cycles when stored at –20°C

Quick-Load Taq 2X Master Mix is also stable for one week at 4°C, so for daily use, an aliquot may be kept at 4°C.

References:

1. Chien, A., Edgar, D.B. and Trela, J.M. (1976) J. Bact., 127, 1550–1557.
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3. Lawyer, F.C. et al. (1993) PCR Methods and Appl., 2, 275–287.
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. Saiki R.K. et al. (1985) Science, 230, 1350–1354.
7. Powell, L.M. et al. (1987) Cell, 50, 831–840.
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Companion Products Sold Separately:

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

Annealing temperature

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