

Taq 5X Master Mix



1-800-632-7799
info@neb.com
www.neb.com



M0285S 013130915031

M0285S



100 reactions (50 µl vol) Lot: 0131309

RECOMBINANT Store at -20°C Exp: 3/15

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).

Taq 5X Master Mix is an optimized ready-to-use solution containing *Taq* DNA Polymerase, dNTPs, MgCl₂, KCl and stabilizers. It is best for routine PCR applications from templates including pure DNA solutions, bacterial colonies, and cDNA. 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
- Colony PCR

Reagents Supplied with Enzyme:

25 mM MgCl₂

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

1X *Taq* Master Mix:

10 mM Tris-HCl (pH 8.6, @ 25°C)
50 mM KCl
1.5 mM MgCl₂
25 units/ml *Taq* DNA polymerase
0.2 mM dNTPs each
5% glycerol
0.08% IGEPAL® CA-630
0.05% Tween® 20

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 [³H]-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 *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' *Taq* 5X 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)
<i>Taq</i> 5X Master Mix	5 µl	10 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 *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 MgCl₂.

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.

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
	60–68°C	5 minutes
Final Extension	60–68°C	5 minutes
Hold	4–10°C	

(see other side)

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: *Taq* 5X Master Mix is stable for fifteen freeze-thaw cycles when stored at -20°C

Taq 5X Master Mix is also stable for six months at 4°C, so for frequent use, an aliquot may be kept at 4°C.

References:

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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.
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7. Powell, L.M. et al. (1987) *Cell*, 50, 831–840.
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Companion Products Sold Separately:

Magnesium Chloride (MgCl₂) Solution
#B9021S 6.0 ml

 Annealing temperature



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