

PCR Protocol for *Taq* DNA Polymerase with Standard *Taq* Buffer (NEB #M0273)

Materials Required but not Supplied

Taq DNA Polymerase with Standard *Taq* Buffer

- Template DNA and associated forward and reverse primers
- Deoxynucleotide (dNTP) Solution Mix (NEB #N0447)
- Nuclease-free Water (NEB #B1500)

Overview

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (1). *Taq* DNA Polymerase is an enzyme widely used in PCR (2). The following guidelines are provided to ensure successful PCR using NEB's *Taq* DNA Polymerase. These guidelines cover routine PCR. Amplification of templates with high GC content, high secondary structure, or low template concentrations, may require further optimization. The NEB [Tm Calculator](#) is highly recommended to estimate an appropriate annealing temperature when using NEB products for PCR.

Protocol

Reaction setup:

1. Assemble all reaction components on ice. Each component should be gently mixed before adding to the reaction in a sterile thin-walled PCR tube. Mix the entire reaction to ensure homogeneity. Collect all liquid to the bottom of the tube with a quick centrifuge spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Component	25 μ l reaction	50 μ l reaction	Final Concentration
10X Standard <i>Taq</i> Reaction Buffer	2.5 μ l	5 μ l	1X
10 mM dNTPs	0.5 μ l	1 μ l	200 μ M
10 μ M Forward Primer	0.5 μ l	1 μ l	0.2 μ M (0.05–1 μ M)
10 μ M Reverse Primer	0.5 μ l	1 μ l	0.2 μ M (0.05–1 μ M)
Template DNA	variable	variable	<1,000 ng
<i>Taq</i> DNA Polymerase	0.125 μ l	0.25 μ l	1.25 units/50 μ l PCR
Nuclease-free water	to 25 μ l	to 50 μ l	

2. Quickly transfer the reactions to a thermocycler preheated to the denaturation temperature (95°C) and begin thermocycling.

Thermocycling Conditions for a Routine PCR:

STEP	TEMP	TIME
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Initial Denaturation	95°C	30 seconds
25-30 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	

*Use of the [Tm Calculator](#) is highly recommended.

General Guidelines:

1. Template:

Use high-quality, purified DNA templates to maximize success with PCR. 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–10 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 can be used to design or analyze primers. The final concentration of each primer in a 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 Standard *Taq* Reaction Buffer 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 (3) or formamide (4).

4. Deoxynucleotides:

The final concentration of dNTPs is typically 200 µM of each deoxynucleotide.

5. *Taq* DNA Polymerase Concentration:

We generally recommend using *Taq* DNA Polymerase at a concentration of 25 units/ml (1.25 units/50 µl reaction). However, the optimal concentration of *Taq* DNA Polymerase may range from 5–50 units/ml (0.25–2.5 units/50 µl reaction) in specialized applications.

6. 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 initial 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.

7. 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. The NEB [Tm Calculator](#) is recommended to calculate an appropriate annealing temperature.

When primers with annealing temperatures above 65°C are used, a 2-step PCR protocol is possible (see #10).

8. 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.

9. Cycle number:

Generally, 25–35 cycles yield sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.

10. 2-step PCR:

When primers with annealing temperatures above 65°C are used, a 2-step thermocycling protocol is possible.

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. The Monarch[®] Spin PCR & DNA Cleanup Kit (5 µg) (NEB #T1130) is recommended as an efficient method for purification and concentration up to 5 µg of high-quality, double-stranded and single-stranded DNA.

Thermocycling conditions for a routine 2-step PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C 65-68°C	15-30 seconds 1 minute/kb
Final Extension	65-68°C	5 minutes
Hold	4-10°C	

References:

1. Saiki R.K. et al. (1985). *Science*. 230, 1350-1354.
2. Powell, L.M. et al. (1987). *Cell*. 50, 831-840.
3. Sun, Y., Hegamyer, G. and Colburn, N. (1993). *Biotechniques*. 15, 372-374.
4. Sarkar, G., Kapelner, S. and Sommer, S.S. (1990). *Nucleic Acids Res.* 18, 7465.

Related Resources

- [Tm Calculator](#)
- [Guidelines for PCR Optimization with *Taq* DNA Polymerase](#)
- [Taq PCR Kit Troubleshooting Guide](#)