NEW ENGLAND Biolabs®

# PCR Using One*Taq*® DNA Polymerase (NEB #M0480)

## Materials Required but not Supplied

One*Taq*® DNA Polymerase

- 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. *Taq* DNA Polymerase is an enzyme widely used in PCR. One*Taq* DNA Polymerase allows for greater amplification sensitivity across a wide variety of amplicons regardless of GC content. The following guidelines are provided to help ensure successful PCR using New England Biolabs' One*Taq* DNA Polymerase. These guidelines cover most routine PCR. Specialized applications may require further optimization.

## 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 spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

| COMPONENT | 25 µl reaction | 50 µl reaction | FINAL CONCENTRATION |
| --- | --- | --- | --- |
| 5X One*Taq* Standard Reaction Buffer* | 5 µl | 10 µl | 1X |
| 10 mM dNTPs (NEB #N0447) | 0.5 µl | 1 µl | 200 µM |
| 10 µM Forward Primer | 0.5 µl | 1 µl | 0.2 µM |
| 10 µM Reverse Primer | 0.5 µl | 1 µl | 0.2 µM |
| One*Taq* DNA Polymerase | 0.125 µl | 0.25 µl | 1.25 units/50 µl PCR** |
| Template DNA | variable | variable | < 1,000 ng |
| Nuclease-free water | to 25 µl | to 50 µl | |

**OneTaq GC Reaction Buffer and High GC Enhancer can be used for difficult amplicons.*

***For amplicons between 3–6 kb, use 2.5–5 units/50 µl rxn.*

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.

2. Quickly transfer the reactions to a thermocycler preheated to the denaturation temperature (94°C) and begin thermocycling.

**Thermocycling Conditions for a Routine PCR:**

| STEP | TEMP | TIME |
|---|---|---|
| Initial Denaturation | 94°C | 30 seconds |
| 30 Cycles | 94°C<br>45-68°C<br>68°C | 15-30 seconds<br>15-60 seconds<br>1 minute per kb |
| Final Extension | 68°C | 5 minutes |
| Hold | 4-10°C | |

**Use of the NEB Tm Calculator is highly recommended.*

# General Guidelines

1. **Template:**
   Use of high-quality, purified DNA templates greatly enhances the success of 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 0.2 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* Standard Reaction Buffer is 1.8 mM. This supports satisfactory amplification of most amplicons. However, $Mg^{++}$ can be further optimized in 0.2 mM increments using $MgCl_2$ (NEB #B9021).

   Amplification of some difficult targets, like GC-rich sequences (50-65% GC), may be improved by the use of One*Taq* GC Reaction Buffer. The final $Mg^{++}$ concentration in 1X One*Taq* GC Reaction Buffer is 2.0 mM. To optimize the $Mg^{++}$ concentration of the One*Taq* GC Reaction Buffer, $MgSO_4$ should be used (NEB #B1003).

   For extremely difficult amplicons (>65% GC), 10–20% One*Taq* High GC Enhancer can be added to reactions with One*Taq* GC Reaction Buffer. The enhancer should not be used alone and typically increases yields when other conditions have failed.

4. **Deoxynucleotides:**
   The final concentration of dNTPs is typically 200 µM of each deoxynucleotide.

5. **One*Taq* DNA Polymerase Concentration:**
Generally, use One*Taq* DNA Polymerase at a concentration of 25 units/ml (1.25 units/50 µl reaction) for amplicons up to 3 kb. The optimal concentration of OneTaq DNA Polymerase may range from 5–100 units/ml (0.25–5 units/50 µl reaction). For specialized applications, including 3–6 kb amplicons, 2.5–5 units/50 µl reaction is recommended. Note that in some cases increasing the amount of enzyme in the reaction can be inhibitory.

6. **Denaturation:**
An initial denaturation of 30 seconds at 94°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 94°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5-minute denaturation at 94°C is recommended to lyse cells.

   During thermocycling, a 15–30 second denaturation at 94°C is recommended.

7. **Annealing:**
The annealing step is typically 15–60 seconds. The annealing temperature is based on the Tm 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 Tm. The NEB $T_m$ Calculator is recommended to calculate an appropriate annealing temperature.

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 68°C are used, a 2-step thermocycling protocol (combining annealing and extension into one step) is possible.

    **Thermocycling conditions for a routine 2-step PCR:**

| STEP | TEMP | TIME |
|---|---|---|
| Initial Denaturation | 94°C | 30 seconds |
| 30 Cycles | 94°C<br>68°C | 15-30 seconds<br>1 minute per kb |
| Final Extension | 68°C | 5 minutes |
| Hold | 4-10°C | |

11. **PCR Product:**
A significant portion of the PCR products generated using One*Taq* DNA Polymerase contain dA overhangs at the 3´end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

# Related Resources

- Tm Calculator