

Protocol for OneTaq[®] 2X Master Mix with Standard Buffer (NEB #M0482)

Materials Required but not Supplied

OneTaq[®] 2X Master Mix with Standard Buffer

- 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 New England Biolabs' OneTaq 2X Master Mix with Standard Buffer. These guidelines cover routine PCR. Amplification of templates with high GC content, high secondary structure or low template concentrations may require further optimization.

Protocol

Reaction setup:

1. Assemble all reaction components. Each component should be gently mixed before adding to the reaction in a sterile thin-walled PCR tube. The entire reaction should be mixed again 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
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
OneTaq 2X Master Mix with Standard Buffer	12.5 µl	25 µl	1X
Nuclease-free Water	to 25 µl	to 50 µl	

2. Transfer PCR tubes from ice to a PCR machine with the block preheated to 94°C and begin thermocycling.

Thermocycling conditions for a routine PCR:

STEP	TEMP	TIME
Initial Denaturation	94°C	30 seconds

STEP	TEMP	TIME
30 Cycles	94°C 45-68°C 68°C	15-30 seconds 15-60 seconds 1 minute/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	

*Use of the [NEB TmCalculator](#) 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 PCR 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* Master Mix with Standard Buffer is 1.8 mM. This supports satisfactory amplification of most amplicons. However, Mg⁺⁺ can be further optimized in 0.2 mM increments using MgCl₂ ([NEB# B9021](#)).

For amplification of difficult targets, like GC-rich sequences, we recommend One *Taq* 2X Master Mix with GC Buffer ([NEB# M0483](#)). Alternatively, DMSO or formamide may be used.

4. 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.

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 [Tm Calculator](#) to determine appropriate annealing temperature 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 yield sufficient product. Up to 45 cycles may be required to detect low copy number targets.

8. **PCR Product:**

A significant portion 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.

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.

References:

1. Saiki, R.K. et al (1985). *Science*. 230, 1350-1354.
2. Powell, L.M. et. al. (1987). *Cell*. 50, 831-840.

Related Resources

- [Tm Calculator](#)