NEW ENGLAND Biolabs®

Protocol for One*Taq*® Hot Start 2X Master Mix with Standard Buffer (NEB #M0484)

Overview

PCR

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' One*Taq* Hot Start 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.

Introduction

Reaction setup:

Due to the presence of the inhibitor, reactions can be assembled on the bench at **room temperature** and transferred to a thermocycler. *No separate activation step is required to release the inhibitor from the enzyme.*

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
One*Taq* Hot Start 2X Master Mix with Standard Buffer	12.5 µl	25 µl	1X
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 to a PCR machine and begin thermocycling:

Thermocycling conditions for a routine PCR:

STEP	TEMP	TIME
Initial Denaturation	94°C	30 seconds
30 Cycles	94°C 45-68°C 68°C	15-30 seconds 15-60 seconds 1 minute per 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. 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* Hot Start 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 $MgCl_2$ (NEB# B9021).

   For amplification of difficult targets, like GC-rich sequences, we recommend One*Taq* Hot Start 2X Master Mix with GC Buffer (NEB# M0485). Alternatively, DMSO (4) or formamide (5) may be used.

4. Denaturation:

   No separate activation step is required to release the hot start inhibitor from the enzyme. An initial denaturation of 30 seconds at 94°C is sufficient to amplify most targets from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 2–4 minutes at 94°C is recommended prior to PCR cycling to fully denature the template. Alternatively, use One*Taq* Hot Start 2X Master Mix with GC Buffer. With colony PCR, an initial 2–5 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. The NEB Tm Calculator is recommended for calculation of an appropriate annealing temperature.

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. 2-step PCR:

   When primers with annealing temperatures of 68°C or above are used, a 2-step thermocycling protocol (combining annealing and extension into one step) is possible.

STEP	TEMP	TIME
Initial Denaturation	94°C	30 seconds
30 Cycles	94°C 65-68°C	15-30 seconds 1 minute/kb
Final Extension	65-68°C	5 minutes
Hold	4-10°C	

9. PCR Product:

   The majority 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.

References:

1. Saiki, R.K. et al. (1994). *Science*. 91, 2216-2220.
2. Powell, L.M. et al. (1987). *Cell*. 50, 831-840.