Protocol for One*Taq* Hot Start DNA Polymerase (M0481)

Overview

PCR

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* Hot Start DNA Polymerase allows for greater amplification sensitivity across a wide variety of amplicons and increased ease of reaction setup. The following guidelines are provided to ensure successful PCR using New England Biolabs' One*Taq* Hot Start DNA Polymerase. These guidelines cover most routine PCR. Specialized applications may require further optimization.

Protocol

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.*

Add to a sterile thin-walled PCR tube:

Component	25 µl reaction	50 µl reaction	Final Concentration
5X One*Taq* Standard Reaction Buffer*	5 µl	10 µl	1X
10 mM dNTPs (#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* Hot Start 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	

*One*Taq* 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.

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	10 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 PrimerSelect™ (DNAStar Inc., Madison, WI) and Primer 3 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* Hot Start 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 $MgCl_2$

4. Deoxynucleotides:

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

5. One*Taq* Hot Start DNA Polymerase Concentration:

   We generally recommend using One*Taq* Hot Start 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 One*Taq* Hot Start DNA Polymerase may range from 5–100 units/ml (0.25–5 units/50 µl reaction) for specialized applications. For 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:

   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. With colony PCR, an initial 2–5 minute incubation at 94°C is recommended to lyse cells.

   During thermocycling a 10–30 second denaturation at 94°C is recommended.

7. Annealing:

   The annealing step is typically 15–60 seconds. 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. We recommend using NEB's Tm Calculator to determine appropriate annealing temperature for PCR.

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

11. PCR Product:

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

Links to this resource

Product Categories: One*Taq*® DNA Polymerases Products, *Taq* DNA Polymerase Products

Applications: Hot Start PCR, Multiplex PCR, Specialty PCR, | More +

Related Products: One*Taq*® Hot Start DNA Polymerase