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PCR Protocol for OneTaq® DNA Polymerase (M0480) V.2

New England Biolabs[1]

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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 ensure successful PCR using New England Biolabs' One *Taq* DNA Polymerase. These guidelines cover most routine PCR. Specialized applications may require further optimization.

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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. OneTaq DNA Polymerase allows for greater amplification sensitivity across a wide variety of amplicons regardless of GC content. The following guidelines are provided to ensure successful PCR using New England Biolabs' OneTaq DNA Polymerase. These guidelines cover most routine PCR. Specialized applications may require further optimization.

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:

A	B
DNA	**Amount**
genomic	1 ng–1 µg
plasmid or viral	1 pg–1 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 Primer3 can be used to design or analyze primers. The final concentration of each primer in a PCR 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 OneTaq DNA Polymerase. The final Mg^{++} concentration in 1X OneTaq 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 MgCl2(NEB #B9021). OneTaq (Mg-Free) Standard Reaction Buffer (NEB #B9024) and supplemental $MgCl_2$ are also available separately for complete control of Mg^{++} concentration in the reaction.

Amplification of some difficult targets, like GC-rich sequences, may be improved by

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the use of OneTaq GC Reaction Buffer. The final Mg^{++} concentration in 1X OneTaq GC Reaction Buffer is 2.0 mM. To optimize the Mg^{++} concentration of the OneTaq GC Reaction Buffer, MgSO4 should be used (NEB #B1003). OneTaq (Mg-Free) GC Reaction Buffer (NEB #B9025) and supplemental MgSO4 are also available separately for complete control of Mg^{++} concentration in the reaction.

For extremely difficult amplicons, 10–20% OneTaq 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:

We generally recommend using OneTaq 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* 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 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 15–30 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 T_m. The NEB T_m Calculator is recommended for calculation of 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.

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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 OneTaq DNA Polymerase contain dA overhangs at the 3´end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

MATERIALS

OneTaq DNA Polymerase - 200 units **New England Biolabs Catalog #M0480S**

Deoxynucleotide Solution Mix - 8 umol of each **New England Biolabs Catalog #N0447S**

Please refer to the Safety Data Sheets (SDS) for health and environmental hazard.

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Set up the following reaction **On ice** :

A	B	C	D
Component	**25 µl reaction**	**50 µl reaction**	**Final Concentration**
5X OneTaq 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
OneTaq 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

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

For amounts of DNA needed, see below:

A	B
DNA	**Amount**
genomic	1 ng–1 µg
plasmid or viral	1 pg–10 ng

2 

Gently mix the reaction.

3 Collect all liquid to the bottom of the tube by a quick spin if necessary and overlay the sample with mineral oil if using a PCR machine without a heated lid.

4 Quickly transfer PCR tubes to a thermocycler preheated to the denaturation temperature (94°C) and begin thermocycling.

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Perform PCR using the general routine below or using your own optimized routine:

A	B	C
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/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	

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