



## Taq Polymerase 2X Mastermix

### INTRODUCTION

The Taq Polymerase 2X Mastermix is a ready-to-use mixture of high quality Taq DNA Polymerase, deoxynucleotides, and reaction buffer in a 2X concentration. It contains all the necessary reagents for amplification of DNA. To set up a PCR reaction: add DNA template, primers and water. PCR products that are amplified up to 6kb in length with Taq DNA Polymerase generate a single base (A) 3' overhang. The error rate of this PCR amplification is  $2.2 \times 10^{-5}$  per nucleotide per cycle.

### SOURCE

A recombinant protein expressed in *E. coli* that carries the *pol* gene for *Thermus aquaticus*.

### ITEM(S) SUPPLIED

	<b>Cat. # 786-449</b>
Taq Polymerase 2X Mastermix	100 reactions

### STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store at  $-20^{\circ}\text{C}$ . Taq Polymerase 2X Mastermix is stable at  $4^{\circ}\text{C}$  for three months or fifteen freeze-thaws. For daily use we recommend storing at  $4^{\circ}\text{C}$ .

### UNIT DEFINITION

One unit (U) of Taq polymerase is defined as the amount of enzyme needed to catalyze the incorporation of 10 nanomoles of deoxyribonucleotides into acid-insoluble material in 30 minutes at  $74^{\circ}\text{C}$ .

### GENERAL PCR GUIDELINES

The following protocol is a general guideline and starting point for PCR amplification, however, as with all polymerases, optimal reaction conditions for incubation times, temperatures and reagent concentrations for all components vary and require precise optimization.

For simple optimization or problematic PCR amplifications we recommend G-Biosciences PCR Optimization Kit (Cat. # 786-418).

#### ❖ Primers:

- Normally 15-30 nucleotides long.
- G-C content of 40-60%.
- Not self-complementary or complementary to other primers in reaction.
- Melting temperature of primer pairs should not differ by  $>5^{\circ}\text{C}$ .

#### ❖ Polymerase:

- Recommend 1-2U polymerase/50 $\mu\text{l}$  reaction. 1U for DNA template less than 10kb and 2U for DNA template greater than 10kb. Higher polymerase concentrations may result in amplification of non-specific products.

#### ❖ PCR Program

- Denaturation
  - 0.5- 2min at  $94-98^{\circ}\text{C}$  is normally sufficient.
- Annealing
  - Optimal annealing temperature is  $\sim 5^{\circ}\text{C}$  lower than the melting temperature of primer-template DNA duplex
- Extension
  - Normally performed at  $72-75^{\circ}\text{C}$ .
  - Extension time is 1 minute for  $<2\text{kb}$  fragments.
  - For larger DNA fragments, increase extension time by 1 minute/kb
- Cycle Number
  - For less than 10 copies of template DNA use 40 cycles
  - If  $>10$  copies, use 25-35 cycles



The PCR reactions should be assembled in a DNA-free environment. We recommend setting up a control reaction that is performed in the absence of DNA to ensure no DNA contamination. Briefly centrifuge the Taq polymerase tubes to collect all the enzyme at the tube bottom.

1. On ice, add the following components to a sterile 0.2ml thin walled PCR tube. For multiple reactions we recommend preparing a master mix.

COMPONENT	Final Concentration	Amount (µl)
Water (sterile)	--	Variable
Primer I (Forward)	0.1-1µM	Variable
Primer II (Reverse)	0.1-1µM	Variable
Taq Polymerase 2X Mastermix		25µl
Template DNA	0.01-1ng of plasmid or phage DNA 0.1-1µg for genomic DNA	Variable
TOTAL VOLUME	--	50µl

2. Mix the contents in the tube and centrifuge briefly.
3. If the thermocycler has a heated lid, proceed with the PCR amplification. If not, overlay the PCR reaction mix with mineral oil or an appropriate amount of wax. Place sample in thermocycler and proceed with amplification.
4. Denature the DNA by heating in a thermocycler for 3 minutes at 94°C
5. Perform ~30 cycles of:
  - a. Denature at 94°C for 30 seconds
  - b. Anneal at 55°C for 30 seconds
  - c. Extend: 72°C for 1min/1kb template
6. Incubate for additional 10 minutes at 72°C and then maintain the reaction at 4°C or store at -20°C until required.

#### **Related Products**

1. **PCR Optimization Kit** (Cat. # 786-418): A straightforward PCR optimization kit for improved standard PCR amplifications and for problematic PCR amplifications.
2. **PCR Enhancer Kit** (Cat. # 786-419): A selection of enhancers to improve PCR amplifications.
3. **GET™ Clean DNA** (Cat. # 786-356): Fast, convenient spin columns for the removal of primers and unincorporated dNTPS from PCR products.
4. **Molecular Grade Water** (Cat. # 786-292): RNase, DNase and protease free water.

**NOTE:** For other related products, visit our web site at [www.GBiosciences.com](http://www.GBiosciences.com) or contact us.