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A Geno Technology, Inc. (USA) brand name

Taq Polymerase 2X Mastermix

(Cat. # 786-449)

INTRODUCTION

2X Taq Mix is a premixed, ready-to-use solution containing Taq DNA Polymerase, dNTPs, Mg^{2+} and Reaction Buffer at optimal concentrations for efficient amplification of DNA templates by PCR. To prepare the final PCR, only primers and template DNA are added. This pre-mixed formulation saves time and reduces contamination due to the fewer pipetting steps required for PCR set up. The mix retains all features of Taq DNA Polymerase.

Taq DNA Polymerase is a thermostable recombinant DNA polymerase derived from thermophilic bacterium *Thermus aquaticus*. Its molecular weight is 94 kDa. Taq DNA Polymerase can amplify DNA target up to 5 kb. The elongation velocity is 0.9~1.2kb/min (70~75°C). It has 5' to 3' polymerase activity but lacks of 3' to 5' exonuclease activity that results in a 3'-dA overhangs PCR product

SOURCE

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

ITEM(S) SUPPLIED (Cat. # 786-449)

Description	Size
Taq Polymerase 2X Mastermix	2 x 1mL
Water, Nuclease Free	2 x 1mL

STORAGE CONDITIONS

Shipped at ambient temperature. Upon arrival, store at -20°C. Taq Polymerase 2X Mastermix is stable at 4°C for three months or fifteen freeze-thaws. For daily use we recommend storing at 4°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°C.

COMPOSITION OF THE TAQ MIX

Taq DNA polymerase is supplied in 2X Taq buffer, 0.4mM dNTPs, 3.2mM $MgCl_2$ and 0.02% bromophenol blue. Taq mix buffer is a proprietary formulation optimized for robust performance in PCR.

PROTOCOL

All solutions should be thawed on ice, gently vortexed and briefly centrifuged.

1. For a total 50µl reaction volume add the following to a thin walled PCR tube on ice

Component of sample	Volume	Final concentration
Taq Mix (2X)	25µl	1X
Forward Primer	variable	0.1-1µM
Reverse Primer	variable	0.1-1µM
Template DNA	variable	10 pg-1µg
Water, nuclease-free	to 50µl	–

Recommendations with Template DNA in a 50µl reaction volume

Human genomic DNA	0.1 µg-1 µg
Plasmid DNA	0.5 ng-5 ng
Phage DNA	0.1 ng-10 ng
E.coli genomic DNA	10 ng-100 ng

2. Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube.
3. Overlay the sample with mineral oil or add an appropriate amount of wax. This step may be omitted if the thermal cycler is equipped with a heated lid.
4. Perform PCR using the following thermal cycling conditions.

Initial Denaturation	94°C	3 minutes
25-35 Cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 1 min
Final Extension	72°C	10 minutes

GENERAL GUIDELINES

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform “no template control” (NTC) reactions to check for contamination

QUALITY CONTROL

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 25µl Taq Mix (2X) with 1µg of pBR322 DNA in 50µl for 4 hours at 37°C and at 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 25µl of Taq Mix (2X) with 1µg of digested DNA in 50µl for 4 hours at 37°C and at 70°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25µl of Taq Mix (2X) with 1µg of E. coli [3H]-RNA (40000cpm/µg) in 50µl for 4 hours at 37°C. 0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25µl of Taq Mix (2X) with 1µg of E. coli [3H]-RNA (40000 cpm/µg) in 50µl for 4 hours at 70°C.

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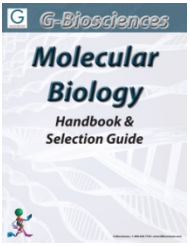

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