G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Taq PLUS Polymerase 2X Mastermix

(Cat. # 786-851)

INTRODUCTION

Plus Mix (2X) is a premixed, ready-to-use solution containing Plus 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. Plus Mix contributes to highly reproducible PCR by reducing the risk of pipetting errors, miscalculation and contamination. It also contributes to higher sensitivity by adding enhancer.

Taq Plus, a mixture of Taq and Pfu polymerase, blends the processivity of Taq with the high fidelity of Pfu. Therefore, this specially formulated Taq plus allows amplification of the higher fidelity and longer templates than the single-enzyme formulations. It is also a better choice for amplifying complex template, such as GC-rich template. And it is suitable as a direct replacement for ordinary Taq Polymerase in most applications. In addition, Using Taq plus results in 3´-dA overhangs PCR products, which can be used in TA clone.

ITEM(S) SUPPLIED (Cat. # 786-851)

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

STORAGE CONDITIONS

It is shipped on blue ice. Upon arrival, store at -20°C. Taq PLUS 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 PLUS 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 PLUS MIX

0.4U/ul Taq Plus DNA polymerase, 2x PCR Buffer, 0.4mM dNTPs, 4mM $MgSO_4$, 0.02% bromophenol blue. Plus 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 PLUS 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

ADDITONAL INFORMATION

    - The half-life of enzyme is >40 minutes at 95°C.
- Taq PLUS DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.
- The error rate of Taq Plus DNA Polymerase in PCR is about $1x10^{-5}$ errors per nucleotide per cycle; the accuracy (an inverse of error rate) an average number of correct nucleotides incorporated before making an error is $3.8x10^{-5}$
- The PCR products are the mixture of 3´-dA overhangs and blunt-ended products. But blunt-ended is the main product.
- •The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.

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 PLUS 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 PLUS 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 PLUS 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 PLUS Mix (2X) with 1µg of E. coli [3H]-RNA (40000 cpm/µg) in 50µl for 4 hours at 70°C.

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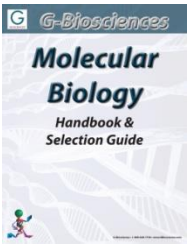

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