BLUETAQ DNA 2X MASTER MIX FS-T-80602

Cat No.	Size
FS-T-80602	5 ML 25 ML

*1 ml (100 PCR reactions of 20 ul vol.)

Description

BlueTaq DNA 2X Master Mix is an optimized PCR premixed solution containing *Taq* DNA Polymerase, dNTPs, $MgCl_2$, KCl, Bromophenol blue tracking dye, and other stabilizers. User only need to add template and primer to complete experiment. The amplified products were directly used for agarose gel electrophoresis.

It is ideally suited to routine PCR applications on various templates including pure DNA solutions, bacterial colony/culture, and cDNA products. It can amplify 5 kb DNA from different sources genomic DNA. It is applicable to PCR reaction, colony PCR identification,rough sample amplification.

Content

Content	FS-T-80602
BLUETAQ DNA 2X Master Mix	5 ml/ 500 reactions

5´-3´exonuclease activity: Yes
3´-5´exonuclease activity: No
Product End: Single-base 3´ Overhangs

Storage Conditions

Store all components at -20˚C in a non-frost-free freezer.

Note

Do not contaminate the Blue Taq DNA 2x Master Mix with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

Quality Control

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

Recommended Protocol

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the Blue Taq DNA 2X Master Mix For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

1. Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.
2. Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.
3. The following table shows recommended component volumes:

Reaction Conditions

Component	20 µl reaction	Final Conc.
BLUETAQ DNA 2X Master Mix	10 µl	1X
10µM Forward Primer	0.2 ~ 2.0 µl	0.1~1.0 µM
10µM Reverse Primer	0.2 ~ 2.0 µl	0.1~1.0 µM
Template DNA	1 ~ 5 µl	< 250 ng
Water, RNase-Free	up to 20 µl	

* **Note:** The optimal reaction concentration varies with different DNA templates.Please refer to the basic principles of PCR below.

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	Imput Amount
Plants, animals and human gDNA	10 ng-100 ng
E.coli, lambda gDNA	100 pg-200 ng
Plasmid DNA	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 Primer3 can be used to design or analyze primers. The final concentration of each primer in a reaction may be 0.05–1 µM, typically 0.1–0.5 µM.

3. Mg2+ and Additives

In the BLUE TAQ DNA 2X Master Mix, the concentration of Mg2+ should be 4 mM, dNTP should be 300 µM. Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO or formamide.

4. Denaturation

An initial denaturation of 3 minutes at 94℃ is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 5 minutes to fully denature the template. With colony PCR, an initial 5 minutes denaturation at 94℃ is recommended to fully decompose the bacteria. During cycling a 10 seconds denaturation at 98℃ is recommended.

5. Extention

The recommended extension temperature is 65℃. The extension time is related to the length of the amplified fragment. Calculate the extension time at the speed of 1 kb/min. A final extension of 5 minutes at 65℃ is recommended.

6. Cycles

Generally, 25–35 cycles yields sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.

PCR Conditions

Step	Temp (˚C)	Time	Cycle
Initial Denaturation	94	3 min.	1
Denature	98	5~ 10 sec.	30
Anneal	55~ 60	20 ~ 30 sec.	
Extend	65	1 kb/min	
Final Extension	65-68	5 min.	1
Hold	4-12° C	-	1

<u>**NOTE**</u>:The recommended pre-denaturation time for colony is :2-5 min

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