LAMDA BIOTECH

PRODUCT INFORMATION

Catalog No.: D152

Product Name: Pfu 2X PCR Master Mix

Size: 5000 µl

Description: Pfu 2X PCR Master Mix is a pre-optimized PCR master mix. It contains the high fidelity ***Pfu DNA polymerase*** in an enhanced PCR reaction buffer in a 2X format. Users only need to add template, primers and H_2O for most PCR experiments. The **Pfu 2X PCR Master Mix** is in green color, containing two non-toxic, inert tracking dyes, blue and yellow, which makes it easy to visualize in every PCR steps, including PCR setup and direct loading of the PCR products for electrophoresis.

Pfu DNA Polymerase is a thermostable DNA polymerase from Pyrococcus furiosus. The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5'->3' direction. Pfu DNA Polymerase also exhibits 3'->5' exonuclease activity, that enables the polymerase to correct nucleotide incorporation errors (proofreading). It has no 5'->3' exonuclease activity. The Pfu DNA polymerase used in this master mix is purified from an *E. coli* strain expressing a *Pfu* DNA Polymerase gene of Pyrococcus furiosus. *Pfu* DNA Polymerase can be used for PCR experiments that require high-fidelity DNA synthesis. The PCR products are blunt ended.

Quality Testing: Pfu 2X PCR Master Mix is a proprietary formulation optimized for robust performance in PCR. All lots of **Pfu 2X PCR Master Mix** have been tested for consistency in PCR experiments using different primers and templates.

Storage: 4°C for up to one month, or -20°C for long term storage.

Unit Definition: One unit incorporates 10nmoles of dNTPs into acid-insoluble material in 30 minutes at 72°C.

***Magnesium Chloride:** In general, 2.0mM $MgCl_2$ is sufficient for most of the PCR experiments. However, this may vary with different conditions and primer sets. Some primers/templates may require adjustments for $MgCl_2$ concentration, which can be achieved as shown below:

Final $MgCl_2$ conc.	Additional 25mM $MgCl_2$ per 50µl reaction
2.0mM	------
2.5mM	1.0µl
3.0mM	2.0µl

Protocol:

1. Setup PCR for a total 50µl reaction volume:

Component	Volume	Final conc.
Pfu 2X Mix	25µl	1X
Forward Primer	variable	0.1-1µM
Reverse Primer	variable	0.1-1µM
Template DNA	variable	10 pg-1µg
Water	to 50µl	–

2. Perform PCR using the following cycles:

Step	Temp.	Duration	Cycles
Initial denature	95°C	3min	1
Denature	95°C	30sec	25-36
Anneal	50-68°C	30sec	
Extension	72°C	60sec/kb	
Final Extension	72°C	10min	1
Storage	4°C	Hold	

3. Technical notes and optimization:

a) For more robust amplification, add addition Pfu DNA polymerase as needed in 0.5 µl increments.

b) Template DNA needed: Genomic: 50-250ng; Plasmid: 1pg-10ng; Viral DNA: 1pg-10ng.

c) For optimization of PCR results, adjust annealing temperature and Mg_2^+ as needed.

This product is for research use only.