Differentiating Research

Product Details

PFU MasterMix (2X)

For High Fidelity Amplifications

Product Overview

This is 2X concentrated solution for PCR reaction which contains dNTPs, Pfu **DNA Polymerase**, Mg+2 and other critical reaction components. It doesn't contain primers and DNA template. Pfu Mastermix is designed for routine PCR, cloning and other applications which require higher fidelity.

Catalog Details

R1220	100 Reactions
R1221	1000 Reactions
R1222	4000 Reactions

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Storage

- 20 ℃

PCR Protocol for templates ≤ 5 KB

The samples must be prepared in sterile DNase Free micro-centrifuge tubes, with the following composition placed on ice

Components	Example for 50µL reaction	Final Concentration
Template DNA	1 µL	10ng - 250 ng
Forward Primer (10µM)	0.5 µL	0.1 - 1µM
Reverse Primer (10µM)	0.5 µL	0.1 - 1µM
DX/DT Pfu MasterMix (2X)	25 µL	1X
Nuclease Free Water	Upto 50 µL	

NOTES:

- Addition of all the above reagents should be done using ice boxes to prevent non-specific amplification.
- To collect all liquid at the bottom of the vial, reaction mix can be kept for a quick spin (10 seconds).
- While doing PCR without heat lid, it is recommended to overlay the sample with 1-2 drops of mineral oil.

Step	Temperature	Time	Cycle
Initial denaturation	95 ± 1 ℃	2 - 5 minutes	1
Denaturation	95 ± 1 ℃	0.5 - 1 minutes	30 - 35
Annealing *	45 - 65 ℃	0.5 - 1 minutes	
Extension**	68 - 72 ℃	1 minute/kb	
Final Extension	68 - 72 ℃	5-10 minutes	1
Hold, if required.	2- 8 ℃	variable	1

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NOTES:

- Addition of all the above reagents should be done using ice boxes to prevent non-specific amplification.
- To collect all liquid at the bottom of the vial, reaction mix can be kept for a quick spin (10 seconds).
- While doing PCR without heat lid, it is recommended to overlay the sample with 1-2 drops of mineral oil.

Step	Temperature	Time	Cycle
Initial denaturation	95 ± 1 ℃	2 - 5 minutes	1
Denaturation	95 ± 1 ℃	≤ 30s	30 - 35
Annealing *	45 - 65 ℃	0.5 - 1 minutes	
Extension**	68 - 72 ℃	1.25 minute/kb	
Final Extension	68 - 72 ℃	10-20 minutes	1
Hold, if required.	2- 8 ℃	variable	1

Quality Control Assays

1. **Purity:** SDS Page analysis with Coomassie Blue Staining resulted in ≥ 95% purity.
2. **Performance testing (1): 2X Mastermix** used to amplify 10ng of DNA template (300bp, 1kb, 3kb and 5kb) in 30 PCR cycles resulted in a single band, confirmed by 1% agarose gel electrophoresis with EtBr.
3. **Performance testing (2)**: **2X Mastermix** was used to amplify 50ng of DNA template (8kb) in 30 PCR cycles resulted in a single band, confirmed by 1% agarose gel electrophoresis with EtBr.
4. **Nuclease tests:** No contamination of endo or exonucleases were detected.

Any Technical Help ?

Please write to us at tech@dxbidt.com . Response can be expected within 24Hrs. Our technical team shall be happy to assist you all the time.

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