Differentiating Research

Product Details

HiFi 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. The mastermix contains Blue dye for direct loading on gel. The dye migrates similar to ~ 500bp fragment on 1% gel for approximate visualisation. Without dye mastermixes are also available.

Catalog Details

R1220	2 ml , DYE+
R1221	10 ml, DYE+
R1220A	2 ml , DYE -

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** - 200 ng
Forward Primer (10µM)	0.5 µL	0.1 - 1µM
Reverse Primer (10µM)	0.5 µL	0.1 - 1µM
DX/DT HiFidelity 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	98℃	30 sec	1
Denaturation	98 ℃	10s	25- 35
Annealing	Tm ℃	20- 30s	
Extension	72 ℃	1 minute/kb	
Final Extension	72 ℃	5-10 minutes	1
Hold, if required.	2- 8 ℃	variable	1

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PCR Protocol for templates > 5KB

Components	Example for 50µL reaction	Final Concentration
Template DNA	1 µL	**50ng** - 200 ng
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DX/DT HiFi MasterMix (2X)	25 µL	1X
Nuclease Free Water	Upto 50 µL	

Step	Temperature	Time	Cycle
Initial denaturation	98 ℃	30s	1
Denaturation	98 ℃	**≤ 20s**	25 - 35
Annealing	Tm ℃	≤30s	
Extension	72 ℃	**1 to 1.25** minute/kb	
Final Extension	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.

CRITICAL NOTES

1. Please follow DX/DT protocols only while doing PCR.
2. Temperature and holding time used in cycles, 98℃ should not be more than what suggested in the protocols.
3. DX/DT Pfu Polymerase is sensitive to very high DNA templates. Don't use more than 200 ng per 50 µl reaction.
4. Extension time and Tm may have to be emperically decised as it changes based on template size.
5. For Site Directed Mutagenesis assays, set up initial trials at 30 Cycles to check for amplification of desired size. After confirmation, re do the PCR at 12 to 18 cycles and use the amplicon for final transformation. As the number of cycles increases, 'mutation rate' may increase. You can also check our ***SITE DIRECTED MUTAGENESIS SERVICES***, for making mutants.

REACH US

Email: info@dxbidt.com | Phone: +91-7349708807 | Web: www.dxbidt.com