Differentiating Research

Product Details

Rigel MasterMix (2X)

For High Fidelity Amplifications

Product Overview

This is 2X concentrated solution for PCR reaction which contains dNTPs, Hi Fidelity DNA Polymerases, Mg+2 and other critical reaction components. It doesn't contain primers and DNA template. Hi Fidelity Rigel Mastermix is designed for routine PCR, cloning and other applications which require higher fidelity. Amplification upto 35KB can be achieved under optimum conditions.

Catalog Details

R3720	1 ml
R3721	5 ml

Storage

- 20 ℃

PCR Protocol

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

Components	Example for 20µL reaction	Final Concentration
(Genomic DNA) / (Plasmid)	1 µL	(10ng - 200 ng)/(100pg-25ng)
Forward Primer (10µM)	1 µL	0.1 - 1µM
Reverse Primer (10µM)	1µL	0.1 - 1µM
Rigel MasterMix (2X)	10 µL	1X
Nuclease Free Water	Upto 20 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.

Thermal cycler guidelines for 3 Step PCR :

Step	Temperature	Time	Cycle
Initial denaturation	95 ℃	1 minute	1
Denaturation	95 ℃	10s	25-30
Annealing *	45 - 60 ℃	20s	
Extension	72 ℃	15s/kb	
Hold, if required.	2- 8 ℃	variable	1

Thermal cycler guidelines for 2 Step PCR : When Tm of primer is more than 68°C

Step	Temperature	Time	Cycle
Initial denaturation	95 °C	1 minute	1
Denaturation	95 °C	10s	30
Annealing+Extention	68 °C	15s/KB	
Hold, if required.	2- 8 °C	variable	1

Quality Control Assays

1. **Purity:** SDS Page analysis with Coomassie Blue Staining resulted in ≥ 95% purity.
2. **Nuclease tests:** No contamination of endo or exonucleases were detected.

Performance Testing

Target sizes tested : λ DNA 25ng was taken from which 15kb fragment was amplified.

Extension time: 15s/Kb

Annealing temperature of all the primers: 58°C

A sharp, single band was observed.

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