LEO™ MasterMix (2X)

For Routine PCR and High GC templates

Product Overview

This is 2X concentrated solution for PCR reaction which contains dNTPs, **Taq DNA Polymerase**, Mg+2 and other critical reaction components. It doesn't contain primers and DNA template. Leo Mastermix is designed for routine PCR and also for amplification of high GC templates.

Catalog Details

R8120	100 Reactions
R8121	1000 Reactions
R8122	4000 Reactions

Visit us for more
www.dxbidt.com

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 50μL reaction	Final Concentration
Template DNA	1 μL	5ng - 100 ng
Forward Primer (10μM)	1 μL	0.1 - 1μM
Reverse Primer (10μM)	1 μL	0.1 - 1μM
Leo MasterMix (2X)	25 μL	1X
Nuclease Free Water	Upto 50 μL	

NOTES:

- 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.

After the preparation of PCR mix samples as above, they are transferred to thermal cycler. The thermal cycling process begins with initial denaturation step. The denaturation process is to ensure complete denaturation of the target DNA at 95℃. The standard steps for thermal cycler are tabulated below with optimum temperature, time, and number of cycles. Generally, 25 – 35 cycles yield sufficient product.

Step	Temperature	Time	Cycle
Initial denaturation	95 ± 1 ℃	2 - 5 minutes	1
Denaturation	95 ± 1 ℃	0.5 - 1 minutes	25 - 35
Annealing *	45 - 65 ℃	0.5 - 1 minutes	
Extension	68 - 72 ℃	1 minute/kb	
Final Extension	68 - 72 ℃	5 - 15 minutes	1
Hold, if required.	2- 8 ℃	variable	1

If your mastermix contains green dye, then load it directly on the gel. The green dye will separate into blue (~4kb) and yellow (~25bp) in 1% agarose gel.

COLONY PCR METHOD

1. Take a pipette tip (10μL) and just touch the colony. Add this colony to LEO mastermix.
2. Follow the PCR program as suggested or your own. Initial denaturation step must be 95℃ for 2 minutes.
3. After PCR, directly load the sample to gel. YOU ARE DONE.

NOTE:

- No need to add entire colony.
- No need to add even half the colony.
- Just a touch is all required.

Quality Control Assays

1. **Purity:** SDS Page analysis with Coomassie Blue Staining resulted in ≥ 95% purity.
2. **Performance testing:** In a 25μL reaction, 12.5μL of mastermix was used to amplify 1ng of DNA template (lambda) of various sizes (300bp, 1kb, 3kb, 5kb) with appropriate primers. PCR was run with 30 cycles resulted in a single band, confirmed by 1% agarose gel electrophoresis with EtBr.
3. **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.

Order Related Queries

Email : info@dxbidt.com | Ph: +91-7349708807
Website: https://dxbidt.com
Address: #87, Dasanapura, Lakshmipura Post, Bangalore - 560073