Differentiating Research

Product Details

LEO MasterMix (2X)

For GC Righ Targets

Product Overview

Mighty Leo Mastermix 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. The pre loaded green dye makes it easier for users to load directly on the agarose gel post PCR.

Features

- Extremely powerful taq based premix, specially optimized for GC rich templates.
- 5X better yield than any standard Taq DNA polymerase.
- Amplification upto 5KB is tested.
- Comes with pre-loaded green dye which separates into blue (Equivalent to ~ 3-5kb DNA fragment) and yellow (Equivalent to ~25bp DNA fragment) on 1% agarose gel.
- Super thermostable: Mastermix is made with proprietary formulation buffer which makes shipping possible at Room Temperature. The mastermix is tested for its massive amplification, even after incubating at 45℃ for at least 7 days !

Catalog Details

R8120	5 ml
R8121	20 ml

PCR Protocol

Components	Example for 50µL reaction	Final Concentration
Template DNA	1 µL	1ng - 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	

Step	Temperature	Time	Cycle
Initial denaturation	95 ℃	2 minutes	1
Denaturation	95 ℃	30s	25 - 35
Annealing *	55 - 65 ℃	30s	
Extension	72 ℃	1 minute/kb	
Final Extension	72 ℃	5 - 15 minutes	1
Hold, if required.	2- 8 ℃	variable	1

Leo mastermix contains green dye hence, you can directly load it directly on the gel. The green dye will separate into blue (3 -5 kb) and yellow (~25bp) in 1% agarose gel.

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COLONY PCR METHOD - DIRECT

NOTE:

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

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

COLONY PCR ALTERNATIVE METHOD

1. Take a pipette tip (10µL) and take 1 full colony(Approximate size of the colony could be 0.5 to 1mm DIA). Add this colony to 00µL of Nuclease Free Water or Autoclaved LB Media*
2. Take 1µL and 5µL from the above and test for PCR SEPARATELY.
3. Follow the PCR program as suggested. Initial denaturation step must be 95℃ for 2 minutes.
4. After PCR, directly load the sample to gel. YOU ARE DONE.

(If you are using media then you can use the 100µL (left out) cell-suspension for taking fresh batch. Nuclease free water may lyse the cells hence you can't use it for taking batch)*

Quality Control Assays

1. **Purity:** SDS Page analysis with Coomassie Blue Staining resulted in ≥ 99% purity for Taq.
2. **Performance testing:** In a 20µL reaction, 10 µL of mastermix was used to amplify 5KB fragment Lambda DNA (25ng) with appropriate primers. PCR was run with 30 cycles resulted in a single band , confirmed by 1% agarose gel electrophoresis with EtBr and also safestain green.
3. **Nuclease tests:** No contamination of endo or exonucleases were detected.

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