

## Product Overview

LEO PRIME<sup>TM</sup> is a Taq-based master-mix (2X) designed for unparalleled performance in routine PCR reactions. With a focus on versatility and robustness, LEO PRIME<sup>TM</sup> stands out with its exceptional features tailored to meet the demands of genotyping, allele specific PCR, low abundance gene testing and multiplexing.

One of its notable attributes is its high PCR inhibitor tolerance, making it particularly adept at handling challenging plant DNA samples containing inhibitors such as phenol, ethanol, IPA, humic acid, Trizol, and EDTA. This capability ensures reliable amplification even in the presence of substances known to impede PCR reactions, guaranteeing accurate and reproducible results even with very crude DNA extracts from plants.

LEO PRIME<sup>TM</sup> can also be used with super precessions for colony PCR screening, where researchers can directly put the colony in the mastermix for PCR reactions. LEO PRIME<sup>TM</sup> Mastermix ensures reliable amplification from cDNA samples, providing researchers with a versatile tool for efficient and accurate gene expression analysis in diverse experimental settings.

## Catalog Details

R8220	4 ml
R8221	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 Prime MasterMix (2X)	25 µL	1X
Nuclease Free Water	Upto 50 µL	

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

Leo Prime 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.

## 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 (10 $\mu$ L) and just touch the colony. Add this colony to LEO mastermix.
2. Follow the PCR program as suggested. Initial denaturation step must be 95°C for 2 minutes.
3. After PCR, directly load the sample to gel. YOU ARE DONE.

## COLONY PCR ALTERNATIVE METHOD

1. Take a pipette tip (10 $\mu$ L) and take 1 full colony (Approximate size of the colony could be 0.5 to 1mm DIA). Add this colony to 100 $\mu$ L of Nuclease Free Water or Autoclaved LB Media\*
2. Take 1 $\mu$ L and 5 $\mu$ L from the above and test for PCR SEPARATELY.
3. Follow the PCR program as suggested. Initial denaturation step must be 95°C 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 100 $\mu$ L (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  $\geq 99\%$  purity for Taq.
2. **Performance testing:** In a 20 $\mu$ L reaction, 10  $\mu$ L of mastermix was used to amplify 1kB fragment (GAPDH gene) from 10 ng of Cotton DNA (CTAB extraction) 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.

## Order Related Queries

## STORAGE

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