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LiDirect™ Genotyping DNA Extraction Buffer

Cat. #: M0014 Size: 2000 rxns

Introduction

LiDirect™ Genotyping DNA Extraction Buffer is designed for fast extraction of tissue DNA for PCR amplification only. The procedure is fast and simple, amenable to automation and does not use hazardous chemicals such as phenol, chloroform or guanidine isothiocyanate. There is no need for mechanical disruption, organic extraction, column purification, or precipitation of the DNA. LiDirect™ Genotyping DNA extraction buffer contains all the reagents needed to rapidly extract genomic DNA from mouse tails mouse tails, yeast, plant cells and other animal tissues, cells, hair shafts, and saliva. The resulting extract can be used directly in PCR reactions using any of our DNA polymerase enzymes and master mixes (M0020-M0025).

Package Information

Components	M0014
LiDirect™ Extraction Buffer*	200 ml
LiDirect™ Stop Solution	200 ml

* **Warning**: Alkaline, corrosive

Storage

Store at 4°C

Protocol

All steps are carried out at room temperature unless otherwise noted. The optimum usage depends on the experiment and specimen, please optimize the sample and PCR template volume by each condition.

1. Place a 1~2 mm piece of mouse tail, or 5~10 mg of tissue, or ~1×10^6 cells into a PCR micro tube.

IMPORTANT: DO NOT add more than 10 mg of tissue per 100 µl of LiDirect™ Extraction Buffer. Too much tissue may inhibit downstream PCR reactions.

2. Add 100 µl of LiDirect™ Extraction Buffer.

3. Incubate at 98°C for 15 min on a thermocycler.

4. Add 100 µl of LiDirect™ Stop Solution.

Note: Tissues will not be completely digested at the end of the incubations. This is normal and will not affect performance.

5. Set up 50 µl of PCR reaction mix and split in half.

6. Take 1 µl of the extract supernatant and mix into the first vial of PCR solution, then take 1 µl of the resulting mix and serial dilute into the second vial.

7. Perform PCR reaction according to the respective manual, and analyze both reactions on an 1-1.5% agarose gel.

Trouble Shooting

No product at all or low yield

1. If positive control works, maybe too much tissue added, perform 1:2 serial dilution with ddH_2O to find out the best dilution factor.
2. Increase PCR cycles.
3. Increase primer concentration.
4. If positive control is not working, perform gradient PCR and increase extension time.
5. Try use LiTaq™ series of enzyme (M0020-M0022) if haven't already done so.

Non-specific products - High molecular weight smears

1. Decrease enzyme concentration.
2. Decrease extension time.
3. Reduce the total number of cycles.
4. Increase annealing temperature or try 2-step protocol.
5. Vary denaturation temperature.
6. Decrease primer concentration.

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