ELK Biotechnology

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Mouse tissue direct PCR kit

Catalog No.	Specification	Storage/Shelf life
EP033-01	50T	-20C°/3 years
EP033-02	200T	-20C°/3 years

Introduction

This kit is specially designed for mouse transgene identification and genotyping. It can directly and rapidly extract genomic DNA from mouse tissue (such as mouse tail, mouse ear, mouse toe, muscle, etc.) for subsequent PCR amplification and detection. The entire process does not require homogenization, disruption, overnight digestion, phenol-chloroform extraction, DNA precipitation or column purification. And the amount of sample required is small, only 5-10 mg mouse tissue or 1-5mm mouse tail can be identified.

The 2xDirect PCR Mix provided by this kit is a PCR master mix with high amplification compatibility, which can perform efficient and specific amplification without completely removing impurities such as proteins. The master mix contains antibody-modified Hot Star Taq DNA Polymerase, dNTPs, Mg2+ and other components, and only needs to add template and primers.

The 2xDirect PCR Mix is pre-mixed with electrophoresis dyes, which can be directly detected by electrophoresis after the reaction is completed. The 3' end of the PCR product has an A, which can be used for TA cloning.

Kit Components

Component	50T	200T
Tissue Lysis Buffer	**1.25 ml*4**	**20 ml**
Digestive Enzyme	**250 μl**	**1 ml**
2x Direct PCR Mix	**500 μl**	**1ml*2**
ddH2O	**1 ml**	**4 ml**
User manual	**1 copy**	**1 copy**

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Advantages

1. Simple and fast: suitable for one-step gene identification from rat tail, rat ear, rat toe and other tissues
2. High specificity: This product uses antibody-modified hot-start Taq enzyme, which has high template affinity and amplification specificity.

Application

1. Mouse transgene identification and genotyping
2. No need to extract genomic DNA, easy to operate

Attentions

1. Cross-contamination between samples should be avoided when taking materials.
2. It is recommended to use fresh animal tissue. If the tissue is frozen for a long time, repeated freezing and thawing should be avoided as much as possible, otherwise the template will be degraded and the PCR efficiency will be affected.
3. The length of the amplified fragment is recommended to be within 2kb for the best amplification efficiency.

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Operation steps

Sample genomic DNA release

1. When using this kit for the first time, please carefully check whether there are crystals in the tissue lysis buffer Tissu Lysis Buffer. If there are crystals, please keep the buffer at room temperature and fully equilibrate until the crystals are completely dissolved, or place it in a water bath at 37°C. to redissolve the precipitate. Dissolve in lysis buffer and store at room temperature.

2. Prepare tissue digest solution according to the formula below

Component	Volume（uL)
Tissu Lysis Buffer	95
Digestive Enzyme	5

Note: Digestive solution is recommended to be fresh prepared before use to ensure the activity of Digestive Enzyme.

3. Take a small amount of tissue sample (about 5~10 mg, about 1~5 mm of rat tail) in a centrifuge tube, add 100uL tissue digestion solution, ensure that the tissue sample is completely infiltrated in the tissue digestion solution, and treat it at 55 °C for 30 minutes. Vortex to mix to improve tissue digestibility.

4. Centrifuge at 12,000 rpm for 2 min, transfer the supernatant to a new centrifuge tube, and store at 4°C or -20°C. The supernatant can also be taken directly for subsequent PCR amplification

PCR identification

When performing PCR identification, it is recommended to set a negative or positive reaction in order to exclude false positive or false negative interference。

1、reaction system

Component	Volume（uL)	Final concentration
2x Direct PCR Mix	10	1x
Forward primer（10uM)	0.5	0.2~0.4 uM
Reverse primer（10uM)	0.5	0.2~0.4 uM

Lysate supernatant (DNA template)	1	-
ddH2O	Make up to 20uL	-

Note:each component should be mixed well repeatedly before use.

a)Amount of template used: It is recommended to take template according to 1-5% of the total system amount.

b) Reaction system: 20ul is recommended, and can also be adjusted according to usage habits.

c) After the preparation of the reaction system is completed, the system needs to be mixed and centrifuged instantly

Amplification program

Cycle steps	Temperature (℃)	Time	Number of cycles
Pre-denaturation	94	1-5 min	1x
Denaturation	94	30 sec	35x
Annealing	60	30 sec	
Extension	72	30 sec/kb	
Final extenstion	72	10 min	1x

Note: a) Annealing temperature: refer to the theoretical Tm value of primers, which can be set 2-5℃ lower than the theoretical value. If the specificity of the amplified product is poor, or the Tm value of the upstream and downstream primers is greatly different, the annealing temperature gradient pre-experiment can be performed first to obtain the optimal annealing temperature.

Amplification product identification

After the reaction, take 6ul of the amplified product and directly perform agarose gel electrophoresis without adding Loading Buffer.