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Mouse Direct PCR Kit Instructions

Composition

Mouse Direct PCR Kit Cat. No.	20 Preps 7812020	200 Preps 7812200	500 Preps 7812500
Lysis Buffer	2 ml	10 ml×2	10 ml×5
Proteinase K	100 µl	1 ml	1.25 ml×2
2×PCR Mix	200 µl	1 ml×2	1 ml×5
ddH_2O	200 µl	1 ml×2	1 ml×5
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Storage

Store at -20℃ (Lysis Buffer can be stored at room temperature) with an expiration date of more than 2 years.

Technical Support

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Introduction

This kit is specially developed for rapid genotype identification in mouse, which can quickly release sufficient amounts of genomic DNA from mouse tail, ears or toes, etc., and the digestion time is only 10 min, without extraction and purification, and the digested product can be directly used as a template for PCR amplification.

The 2× PCR Mix provided with this kit is an optimized two-fold concentration PCR master mix with Taq Plus DNA polymerase, PCR enhancer, and protein stabilizer synergistically improving PCR efficiency and sensitivity. The amplified product of interest has an A base attached to the 3′ end and can be cloned directly in T-Vector. The product contains a blue electrophoresis indicator dye, and the amplified product can be detected directly by agarose electrophoresis.

Equipment And Reagents to Be Supplied by Users

1. 1.5 ml centrifuge tubes, pipette tips, disposable gloves, and protective equipment.
2. Microcentrifuge (s) (with rotor for 1.5 ml and 2 ml tubes).
3. Water bath or dry bath.

Preparation Before Use

1. Set the water bath or dry bath temperature to 56℃ and 95℃.
2. Collect samples, and the reference range for the sample size of different tissue samples of mouse was as follows:

Sample type	Sample size
Tail or toes	1-2 mm or 3-5 mg
Ear	<5 mm^2 or 3–5 mg
Organ	<20 mm^2

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Protocol

1. Tissue digestion

(1) Tissue digests are prepared according to the number of samples, and the reagent ratios are as follows:

Reagent	Single sample
Lysis Buffer	100 µl
Proteinase K	5 µl

The tissue digestion solution is prepared and mixed well to use at once.

(2) Add 100 µl tissue digestion solution to each 1.5 ml centrifuge tube containing mouse tissue samples, be sure to completely submerge the tissue in the solution, incubate at 56℃ for 10 min.

* There is no need to worry if the tissue is still relatively intact after digestion, as sufficient genomic DNA has been released and will not affect subsequent PCR experiments.
* If DNA is extracted from mouse tissues older than 3 months, the volume of digested fluid can be increased to 200 µl or the digestion time can be extended appropriately.
* If the gene of interest is difficult to amplify, it is recommended to extend the digestion time to 30 min.

(3) Spin down the liquid on the lid to settle to the bottom of the tube, incubate at 95℃ for 5 min.

* Do not omit the step of spin down, otherwise the residual proteinase K on the lid is not inactivated at 95℃ and may affect subsequent PCR amplification.

(4) Centrifuge at the full speed (≥13,000 rpm) for 5 min and take the supernatant as a PCR template. The digested supernatant can be stored at -20℃ for three months.

* In order to ensure PCR amplification efficiency, especially for difficult-to-amplify genes, PCR templates should be used as soon as possible.

2. PCR Amplification

(1) Thaw 2× PCR Mix, ddH_2O, template DNA, and primers at room temperature and place on ice.

(2) After thawing, invert the components several times to mix evenly, and the PCR reaction was prepared according to the following compositions:

2×PCR Mix	10 µl	25 µl
Forward primer (10 µM)	0.5 µl	1 µl
Reverse primer (10 µM)	0.5 µl	1 µl
template	1 µl*	2 µl*
ddH2O	8 µl	21 µl
Total	20 µl	50 µl

* The amount of template can be increased or decreased appropriately to increase the brightness of the band of interest or to reduce the amplification of non-specific fragment.

(3) Flick the PCR reaction tube to mix well and centrifuge briefly.

(4) Example of a PCR reaction program setup

94℃ 3 min
94℃ 30 sec
※55℃ 30 sec
§ 72℃ 1 min
} 35 Cycles
72℃ 5 min

※The actual optimal annealing temperature shall prevail.
§ Calculated at 2 kb/min.

3. Result detection

5-10 µl of the amplification product was directly detected by agarose gel electrophoresis.

Relationship between agarose gel concentration and optimal resolution range for linear DNA:

Agarose concentration	Optimal linear DNA resolution range
	1,000~30,000
0.7%	800~12,000
1.0%	500~10,000
1.2%	400~7,000
1.5%	200~3,000
2.0%	50~2,000