TransDirect® Mouse Genotyping Kit

Cat. No. AD501

Storage: at -20°C for two years

Description

TransDirect® Mouse Genotyping Kit uses a unique lysis buffer to prepare mouse genotyping PCR-ready DNA from fresh or frozen mouse tissue slices, such as mouse ears, toes and tails. The high-efficient 2×*TransDirect*® Mouse Genotyping SuperMix (+dye) can effectively suppress the inhibitory activities of the crude lysate for PCR amplification.

Applications

- Direct PCR amplification from crude lysate.
- Suitable for PCR-based rapid mouse genotyping.
- Easy-to-use and suitable for high-throughput applications.
- Suitable for multiplex PCR with up to 5 pairs of primers.

Kit Contents

Component	AD501-01 (100 rxns)	AD501-02 (500 rxns)
AD1 Buffer	4 ml	20 ml
AD2 Buffer	1 ml	5 ml
2×*TransDirect*® Mouse Genotyping SuperMix (+dye)	1 ml	5×1 ml
10×GC Enhancer	1 ml	5×1 ml
ddH_2O	5 ml	25 ml

Procedures

A: Template preparation

1. Add 40 µl of AD1 buffer and 10 µl of AD2 buffer to a 0.2 ml PCR tube. For more samples, premix AD1 buffer with AD2 buffer at a ratio of 4:1. The mixture can be stored up to 2 hours at room temperature.
2. Add mouse tissue to the mixture of AD1/AD2, mix thoroughly by pipetting up and down.
   Ear tissue: A diameter of 3 mm round by punching or approximate size by cutting
   Toe tissue: About 2 mm (NOT including nail)
   Tail tissue: About 2 mm tail tip
3. Transfer the PCR tube in a PCR thermal cycler (with a heating lid), incubate at 55°C for 10 minutes, then at 95°C for 3 minutes.
4. The obtained lyaste can be used as PCR template directly or stored at 4°C for three months or at -20°C for six months.

B: PCR amplification

Reaction Components

Component	Volume	Final Concentration
Unpurified Lysate	0.5-4 µl	-
Forward Primer (10 µM)	0.4 µl	0.2 µM
Reverse Primer (10 µM)	0.4 µl	0.2 µM
2×*TransDirect*® Mouse genotyping SuperMix (+dye)	10 µl	1×
ddH_2O	Variable	-
Total volume	20 µl	-

Thermal cycling conditions

94°C	5-10 min	
94°C	30 sec	35-40 cycles
50-60°C	45 sec	
65°C	0.5-1 kb/min*	
65°C	5-10 min	

* For multiplex PCR, suggest to use the extension time (at a rate of 0.5 kb / min) according to the longest fragment.

Notes

- Completely thaw the contents in the tube and mix well before use.
- Don't use too many tissues to avoid incomplete lysis.
- If needed, store AD1 Buffer at 4°C to avoid repeated freezing and thawing (up to 1 month).
- Although 1μl or 2 μl of lysate used as PCR template can usually get good results, the amount of templates is suggested to be optimized.
- For a better result of multiplex PCR, besides adjusting annealing temperature, template amount, primer ratio, a final concentration of 0.5-2×GC enhancer can be added.

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