

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 | 12 ml | 60 ml |
| AD2 Buffer | 3 ml | 15 ml |
| 2×TransDirect® Mouse Genotyping SuperMix (+dye) | 1 ml | 5×1 ml |
| 10×GC Enhancer | 1 ml | 5×1 ml |
| Nuclease-free Water | 5 ml | 25 ml |

Procedures

A: Template preparation

- 1. Add 120 µl of AD1 buffer and 30 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 2-8°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 μΜ |
| Reverse Primer (10 μM) | 0.4 μl | 0.2 μΜ |
| 2×TransDirect® Mouse Genotyping SuperMix (+dye) | 10 µl | 1× |
| Nuclease-free Water | Variable | - |
| Total volume | 20 μl | - |





Thermal cycling conditions

| 94°C | 5-10 min | |
|---------|---------------|--------------|
| 94°C | 30 sec | |
| 50-60°C | 45 sec | 35-40 cycles |
| 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 2-8°C to avoid repeated freezing and thawing (up to 1 month).
- Although $1\mu l$ or $2\mu l$ 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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