

# TransDirect® Mouse Genotyping Kit

Please read the manual carefully before use

Cat. No. AD501

Storage: at -18°C or below 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.  
Note: If the sample is not minced or the sample amount is large, the incubation time at 55°C may be appropriately extended, but should not exceed 2 hours.
4. The obtained lysate can be used as PCR template directly or stored at 2-8°C for short-term storage or at -20°C for long-term storage.

### 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×
Nuclease-free Water	Variable	-
Total volume	20 µl	-



#### Thermal cycling conditions

94°C	5-10 min	} 35-40 cycles
94°C	30 sec	
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

- Don't use too many tissues to avoid incomplete lysis.
- The lysate can be stored at 2-8 °C for up to three months, and at -20 °C for approximately six months.
- If needed, store AD1 Buffer at 2-8°C to avoid repeated freezing and thawing (up to 1 month).
- For closely sized multiplex PCR products, it is recommended to use a high-percentage agarose gel for electrophoresis.
- Although 1 µl 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.

**For research use only, not for clinical diagnosis.**

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