FISHER MOLECULAR BIOLOGY

DE-736 MOUSE TISSUE DIRECT PCR KIT

Product Components

Components	Size-1 100 RXN(25 μL/RXN)	Size-2 5 × 100 RXN(25 μL/RXN)
Hot Start 2X PCR Mix for Mouse Genotyping	1.25 mL	100 RXN × 5
Tissue Lysis Buffer II*	20 mL	

*Note：Tissue Lysis Buffer II contains proteinase K, which can be stored at 4 ℃/room temperature for less than 4 weeks, -20 ℃ is recommended if stored for more than 4 weeks.

Product Description

Mouse Tissue Direct PCR Kit is specially designed for the rapid genotyping of mouse, which contains Tissue Lysis Buffer II and HS 2X PCR Mix for Mouse Genotyping for DNA release and PCR amplification. This kit can be used for the rapid extraction of genomic DNA from mouse tails, ears, toes, and other tissues. The extracted genomic DNA can be used directly as template for PCR amplification, which greatly shortens the experimental time. During use, immerse the tissue into Lysis Buffer, incubate at 55℃ for 15 min, and then heat at 95℃ for 5 min to inactivate Proteinase K. After centrifugation, the lysate can be directly used as the template for PCR amplification. After repeated tests, it is widely applicable to the amplification of target fragments within 4 kb, and is suitable for Multiplex PCR within four fragments, and optimized conditions are required for fragments above 4 Kb.

Tissue Lysis Buffer II in this kit already contains proteinase K, which does not require additional preparation, and Tissue Lysis Buffer II has been tested to be stable storage for 4 weeks at room temperature or 4 ° C, thus avoiding repeated freeze-thaw cycles.

HS 2X PCR Mix for Mouse Genotyping in this kit contains high-performance DNA Polymerase, dNTP, and an optimized buffer system. It only needs to add primers and templates to perform amplification, thereby reducing pipetting operations, significantly controlling cross-contamination among samples, and improving detection throughput and reproducibility of results.The amplification system contains protective agents that keep HS 2X PCR Mix for Mouse Genotyping stable in activity after repeated freezing and thawing. HS 2X PCR Mix for Mouse Genotyping contains loading buffer, PCR products can be directly loaded for electrophoresis after the reaction.

PCR Product End

Blunt end

Storage: -20℃

Operation Description

Standard Protocol

1. Take a clean 1.5 mL EP tube and add mouse tail to the EP tube (1-2 mm or 3-5 mg is recommended for mouse tail tip and toes, and 1-5 mm^2 or 3-5 mg for mouse ears).
2. Add 200 μL of Tissue Lysis Buffer II to the EP tube to completely submerge the tissue.

Note: Tissue Lysis Buffer II must be fully dissolved till becoming clear before use.

3. The recommended lysis reaction conditions are as follows:

Step	Temp	Time
Digestion	55℃	15 min
Inactivation	95℃	5 min

Note: To ensure the efficiency of DNA release, be sure to immerse all tissues in the lysis buffer. After the incubation, the tissue block may not be completely digested, which is normal and does not affect the use.

4. Vortex lysates to mix thoroughly, then centrifuge at 12,000 rpm for 2 min. Take the supernatant for PCR amplification, or transfer the supernatant to another sterilized EP tube, which can be stored at -20°C for at least three months.

Note: When the supernatant is taken out from -20°C for use, it must be fully dissolved till becomeing clear before use.

Recommended Reaction:

Component	25 μL Reaction	Final Concentration
HS 2X PCR Mix for Mouse Genotyping*	12.5 μL	1X
Forward Primer (10 μM)	0.5 μL	0.2 μM
Reverse Primer (10 μM)	0.5 μL	0.2 μM
Lysate	1-2 μL	/
Nuclease-free Water	to 25 μL	N/A

*Note: HS 2X PCR Mix for Mouse Genotyping contains loading buffer, the PCR products can be used directly for agarose gel electrophoresis, without addition of DNA Loading Buffer.

Recommended PCR Program：

Step	Temp	Time	Cycles
Initial Denaturation	98℃	45 s	1
Denaturation	98℃	10 s	30-35
Annealing	55-65℃	20-30 s	
Extension	72℃	30-60 s/kb*	
Final Extension	72℃	1-5 min	1
Hold	4-12℃	-	1

*Note: Fragments below 4 kb are amplified at 30 s/kb, and fragments above 4 kb are amplified at 60 s/kb.

FAQs

1. No amplification products or low yield:

a. Insufficient lysis and not enough DNA is obtained: Try to extend the 55℃ incubation time to 3 h.

b. PCR inhibitors in the tissue are mixed into the lysate: Try to dilute the lysate by 2-5 times before performing PCR amplification.

c. Proteinase K is not fully inactivated: Try to extend the inactivation time to 30 min.

d. Amplified fragments are relatively long: Set the amplification speed to 1 kb/1 min while extending the final extension time to 10 min, or amplify the fragment in a two-step process.

e. PCR primer error: Set up positive control reaction.

2. Nonspecific products

a. Prepare PCR system at high ambient temperature: The preparation of the reaction system on ice, and the PCR reaction is started as soon as possible.

b. In PCR system, the primer concentration or DNA template concentration is too high: Appropriately reduce the amount of primers and templates.

c. The annealing temperature is too low, or the number of cycles is too high: Try to increase the annealing temperature, or reduce the number of cycles, you can perform gradient optimization experiments.

d. Serious mismatch of PCR primers: Redesign the primers.

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