Ver. HB230603

Mouse Tissue Direct PCR Kit (With Dye)

Product description

This kit can directly and quickly conduct PCR amplification of mouse tissue (such as mouse tail, mouse ear, mouse toe, muscle, etc.), and has strong sample compatibility. Equipped with a powerful lysis buffer, this kit can rapidly lyse samples and release genomic DNA. The lysate can be directly added to the PCR reaction system without purification, and the operation is convenient. In addition, this kit requires low sample input, and 5 mg mouse tissue or 1-5 mm mouse tail can be used for experiments.

The 2× Mouse Direct PCR Mix provided by this kit is a hot-start PCR reaction solution with a 2-fold concentration. It contains all the components used for PCR amplification except the template and primers, which greatly simplifies the operation process and reduces the chance of contamination. The kit can be used for transgene identification, mouse genotyping, etc.

Components

Components No.	Name	10185ES50	10185ES70
10185-A	Buffer ML	5×1 mL	20×1 mL
10185-B	Buffer MT	0.6 mL	2×1.25 mL
10185-C	2× Mouse Direct PCR Mix	500 μL	2×1 mL

a) Buffer ML is a lysis buffer containing strong protein denaturants, please wear gloves.

b) Buffer MT is a stop buffer used to stop the lysis function of Buffer ML.

c) 2× Mouse Direct PCR Mix: Contains hot-start Taq DNA polymerase, dNTP mix, $MgCl_2$, reaction buffer, PCR reaction enhancer, optimizer, stabilizer, electrophoresis indicator dye, etc.

Specifications

Product specification	Kit
Hot start	Built-in Hot Start
Overhang	Blunt
Conditions for transportation	Ice Packs
Product type	Direct PCR Kit
Apply to (application)	Mouse tail, Mouse ear, Rat toe, Viscera, Skin, etc.

Storage

1. Component A: The product should be stored at 2-8°C for one year. For multiple use for a long time, please avoid cross-contamination.

2. Component B/C: The product should be stored at -25°C ~ -15°C for one year. Please avoid repeated freeze-thaw.

Instructions

1. Sample genomic DNA release

1.1 Cut 5-10 mg of animal tissue or 1-5 mm of rat tail and put them in a 1.5 mL centrifuge tube.

1.2 Add 90 μL of Buffer ML to the above centrifuge tube, vortex gently so that the sample is completely infiltrated by the lysate, and centrifuge briefly.

1.3 Incubate at 95°C for 15 min in a constant temperature incubator.

1.4 Add 10 μL Buffer MT, flick and mix to stop lysis.

1.5 Optional steps: centrifugation at 12000 rpm for 2 min.

1.6 Transfer the supernatant to a new centrifuge tube and store at 4°C or -20°C or directly take the supernatant for subsequent PCR amplification.

[Note]: The tissue should be chopped as little as possible to allow the lysis reaction to proceed more smoothly.

[Note]: Incubate at 95°C, usually 15 min to meet most PCR needs. If a larger amount of DNA is required or the sample is difficult to lyse, the time can be extended to 30 min. The tissue mass does not need to be completely lysed. The residual fraction can be removed in a subsequent centrifugation step.

2. Reaction System

Components	Volume (μL)	Final concentration
2× Mouse Direct PCR Mix	10	1×
Forward Primer (10 μmol/L)	0.5	0.2-0.4 μmol/L
Reverse Primer (10 μmol/L)	0.5	0.2-0.4 μmol/L
Lysate product (DNA template)	1	-
ddH_2O	to 20	-

[Note]: All components should be thoroughly mixed before use.

2.1 Template usage: It is recommended to use 1-10% of the total system amount of template, and 1 μL supernatant is recommended as template for 20 μL system.

2.2 Primer final concentration: it is recommended to use 0.2-0.4 μmol/L of primer final concentration to get better results. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.1-0.5 μmol/L.

2.3 Reaction system: 20 μL is recommended, and the volume of the system can also be adjusted according to usage habits.

2.4 System preparation: Prepare the PCR reaction system, place it on a vortexer, vortex and mix, and centrifuge briefly to collect the reaction solution at the bottom of the tube.

3. Reaction program

Cycle step	Temperature	Duration	Cycles
Initial denaturation	94°C	5 min	1
Denature	94°C	10 sec	35
Anneal	60°C	20 sec	
Extend	72°C	30 sec/kb	
Terminal extension	72°C	5 min	1

[Note]:

3.1 Annealing temperature: please refer to the theoretical Tm value of the primer. The annealing temperature can be set 2-5°C lower than the theoretical value of the primer.

3.2 Extension time: please set as 30 sec/kb.

3.3 Number of amplification cycles: 35 cycles can amplify enough products.

3.4 Sample loading by electrophoresis: take 3-5 μL of amplification product and load it.

4. Control response

In the analysis of PCR results, whether positive or negative, the reliability of the results cannot be determined without a control reaction. In order to facilitate the analysis of subsequent experimental results, it is recommended to set positive and negative PCR control reactions during PCR in order to exclude false positive or false negative interference.

Notes

1. To avoid sample to sample cross contamination, the edge of the sampling equipment or the site in direct contact with the sample was immersed in 2% sodium hypochlorite solution after each sampling session, washed repeatedly several times, and then blotted with clean paper towels to dry the residual liquid before use. For the convenience of the test, multiple sampling equipment can also be prepared and cleaned uniformly after use to ensure that each individual sample uses a non-polluting sampling equipment.
2. It is recommended to use freshly collected animal tissue. If it is a long-term frozen tissue, repeated freezing and thawing should be avoided as much as possible, otherwise the template will be degraded and the PCR efficiency will be affected.
3. It is recommended to amplify the fragment length within 1 kb for the best amplification efficiency.
4. For your safety and health, please wear lab coats and disposable gloves for operation.
5. This product is for research use ONLY!