Mouse Tail Genomic DNA Extraction Kit

Project number: M665559

Storage conditions: room temperature.

Products content

Component	M665559 50preps
BufferGTT	15mL
BufferGL	15mL
BufferGW1 (concentrate)	13mL
BufferGW2 (concentrate)	15mL
BufferGE	15mL
ProteinaseK	1.25mL
SpinCoLumnsDMwithCoLLectionTubes	50

Product Introduction

This kit is suitable for extracting high-purity total DNA from fresh or frozen mouse or rat tails. The kit provides a simple and easy-to-use method that eliminates the need for phenol or chloroform extraction during the purification process, and allows for DNA fragments up to 50kb to be obtained, as well as efficient recovery of fragments up to 100bp. The kit adopts a unique lysis solution to effectively lyses the mouse tail samples. The optimized buffer system enables the DNA generated after lysing the mouse tail to be efficiently bound to the silica matrix adsorption column, while other contaminants can flow through the membrane; the inhibitors of PCR and other enzymatic reactions can be effectively removed through a two-step washing step, and finally eluted with a low-salt buffer or water, which results in high purity of the DNA. The purified DNA can be directly used in downstream experiments such as digestion, PCR, ReaL-TimePCR, library construction, SouthernBLot and molecular labeling.

Self-contained reagent: anhydrous ethanol.

Pre-experiment Preparation and Important Notes

1. Repeated freezing and thawing of the sample should be avoided, as this may result in smaller fragments of extracted DNA and a decrease in the amount extracted.

2. Anhydrous ethanol should be added to BufferGW1 and BufferGW2 according to the instructions on the label of the reagent bottle before first use.

3. Before use, please check whether BufferGL appears to be crystallized or precipitated, if there is any crystallization or precipitation, please

re-dissolve BufferGL in 56℃ water bath.

Operation steps

1. Take a section of rat or two sections of mouse tail with a length of 0.4-0.6cm, grind it into fine powder in liquid nitrogen or cut it into pieces and put it into a centrifuge tube (self-prepared).

Add 180 μLBufferGTT and mix with shaking.

Note: Make sure that the starting amount of tissue does not exceed the recommended range.

2. Add 20μL of ProteinaseK, vortex and shake, mix thoroughly.

3. Place in a water bath at 56° C until the tissue solution is completely clear. 6-8 hours of digestion is normally required, vortexing and shaking during incubation to disperse the sample evenly.

Note: 1) If gelatinous material remains after incubation and vortex shaking, overnight digestion or addition of another 20 μL of ProteinaseK for digestion, if necessary, will not affect subsequent operations.

(2) If RNA removal is required, add 4 μL of 100 mg/mL RNaseA solution after the above steps are completed, shake and mix, and leave at room temperature for 5-10 minutes.

4. Centrifuge at 12,000 rpm (~13,400 × g) for 1 min to remove undigested tissues similar to mouse hair, etc. Transfer the supernatant to a new centrifuge tube (self-contained).

5. Add 200μL of BufferGL, vortex and shake, mix well. Add 200μL of anhydrous ethanol, vortex and shake, mix well. Centrifuge briefly so that the solution on the wall of the tube is collected to the bottom of the tube.

Attention:

1) Vortex and shake to mix immediately after adding BufferGL and anhydrous ethanol.

2) If multiple samples are manipulated together, BufferGL and anhydrous ethanol can be mixed in equal proportions and added to the samples together.

3) The addition of BufferGL and anhydrous ethanol may produce a white precipitate that will not affect subsequent experiments.

6. Add all of the solution obtained in step 5 to the adsorption column (SpinCoLumnsDM) that has been loaded into the collection tube, and if the solution cannot be added all at once, it can be transferred in several times. centrifuge at 12,000 rpm for 1 minute, pour out the waste solution in the collection tube, and put the adsorption column back into the collection tube.

7. Add 500 μLBufferGW1 to the adsorption column (check that anhydrous

ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

8. Add 500 μLBufferGW2 to the adsorption column (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

Note: Step 8 can be repeated if further DNA purity is required.

9. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the adsorption column at room temperature for several minutes to dry thoroughly.

Note: The purpose of this step is to remove residual ethanol from the adsorption column; ethanol residue can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).

10. Place the adsorption column in a new centrifuge tube (self-provided), add 50-200 μL of BufferGE or sterilized water to the middle of the adsorption column overhanging the column, leave it at room temperature for 2-5 minutes, centrifuge at 12,000 rpm for 1 minute, collect the DNA solution, and store the DNA at -20℃.

Attention:

(1) If the downstream experiment is sensitive to pH or EDTA, it can be eluted with sterilized water. The pH of the eluent has a great influence on the elution efficiency, if water is used as the eluent should ensure that its pH is 7.0-8.5 (you can use NaOH to adjust the pH of the water to this range), the elution efficiency is not high when the pH is lower than 7.0.

2) Incubation at room temperature for 5 minutes prior to centrifugation increases yield.

3) Re-elution with an additional 50-200 μLBufferGE or sterilized water can increase the yield.

4) If the final concentration of DNA is to be increased, the DNA eluate obtained in step 10 can be re-spiked onto the adsorbent membrane and step 10 can be repeated; if the elution volume is less than 200 μL, the final concentration of DNA can be increased, but the total yield may be reduced. If the amount of DNA is less than 1 μg, it is recommended to elute with 50 μL BufferGE or sterilized water.

5) Because DNA preserved in water is subject to acidic hydrolysis, for long-term storage, elution with BufferGE and storage at -20° C are recommended.