Mouse Tail Genomic DNA Kit (purified lysis) (50 preps) Cat. #: W2094

You can also order from Fisher Sci., and contact them for Cat.#.

Store and ship at room temperature.

Product Description (This product is for research use only.)

This kit is suitable for extracting high-purity total DNA from fresh or frozen mouse or rat tails. The method provided by this kit is simple and easy to perform, and the purification process does not require phenol or chloroform extraction. It can recover DNA fragments up to 50 kb in size, while effectively recovering fragments as small as 100 bp. The kit uses a unique lysis buffer that effectively lyses tail tissue samples, and the optimized buffer system enables the DNA generated from the lysed tail to efficiently bind to the silica-based adsorption column, while other contaminants pass through the membrane. PCR and other enzymatic reaction inhibitors are effectively removed through a two-step wash process. The DNA is eluted using a low-salt buffer or water, resulting in high-purity DNA. The purified DNA can be directly used in downstream applications such as digestion, PCR, Real-Time PCR, library construction, Southern Blot, molecular markers, and more.

User-supplied reagent: Anhydrous ethanol.

Product Components (for 50 preps)

Components	Amount	Components	Amount
Buffer GTT	15 ml	Buffer GE	15 mL
Buffer GL	15 ml	Proteinase K	1.25 mL
Buffer GW1 (concentrate)	13 ml	Spin Column DM with Collection Tube	50 ea.
Buffer GW2 (concentrate)	15 ml		

Preparation Before the Experiment and Important Notes:

1. Samples should avoid repeated freeze-thaw cycles, as this can lead to smaller DNA fragments and reduced extraction yield.
2. Before first use, add anhydrous ethanol to Buffer GW1 and Buffer GW2 according to the instructions on the reagent bottle labels.
3. Before use, check if Buffer GL has crystallized or precipitated. If crystallization or precipitation is observed, dissolve Buffer GL by incubating it in a 56°C water bath.

Protocol

1. Take a segment of a rat tail (or two segments of a mouse tail), each 0.4-0.6 cm in length. Grind the tail into a fine powder in liquid nitrogen or cut it into small pieces and place it into a centrifuge tube (user-supplied). Add 180 µL of Buffer GTT and vortex to mix thoroughly.

Note: Ensure the amount of tissue does not exceed the recommended range.

2. Add 20 µL of Proteinase K, vortex, and mix thoroughly.

3. Incubate in a 56°C water bath until the tissue solution becomes completely clear, which typically takes 6-8 hours. During incubation, vortex occasionally to ensure even dispersion of the sample.

Note: 1) If gel-like substances remain after incubation and vertexing, continue digestion overnight or add another 20 µL of Proteinase K if necessary. This will not affect the subsequent steps.

2) To remove RNA, after completing the above step, add 4 µL of 100 mg/mL RNase A solution (Product No: CW0601S), mix thoroughly by vertexing, and leave at room temperature for 5-10 minutes.

4. Centrifuge at 12,000 rpm (~13,400×g) for 1 minute to remove undigested tissue such as mouse hair. Transfer the supernatant to a new centrifuge tube (user-supplied).

5. Add 200 µL of Buffer GL and vortex thoroughly to mix. Then add 200 µL of anhydrous ethanol and vortex again to mix thoroughly. Briefly centrifuge to collect any liquid on the walls of the tube. **Note**:

1. After adding Buffer GL and anhydrous ethanol, immediately vortex to mix thoroughly.
2. If processing multiple samples at once, Buffer GL and anhydrous ethanol can be premixed in equal proportions and added to the samples together.
3. A white precipitate may form after adding Buffer GL and anhydrous ethanol, which will not affect the subsequent experiments.

6. Transfer the entire solution from step 5 into a spin column (Spin Columns DM) placed in a collection tube. If the solution cannot be added all at once, transfer it in multiple steps. Centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and place the spin column back into the collection tube.

7. Add 500 µL of Buffer GW1 (check if anhydrous ethanol has been added beforehand) to the spin column. Centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and place the spin column back into the collection tube.

8. Add 500 µL of Buffer GW2 (check if anhydrous ethanol has been added beforehand) to the spin column. Centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and place the spin column back into the collection tube. **Note**: If higher DNA purity is needed, step 8 can be repeated.

9. Centrifuge at 12,000 rpm for 2 minutes and discard the waste liquid in the collection tube. Let the spin column sit at room temperature for a few minutes to air dry completely. **Note**: This step is crucial to remove any residual ethanol from the spin column, as ethanol residues can affect subsequent enzymatic reactions (e.g., digestion, PCR).

10. Place the spin column into a new centrifuge tube (user-supplied). Add 50-200 µL of Buffer GE or sterile water to the center of the spin column membrane, let it sit at room temperature for 2-5 minutes, and then centrifuge at 12,000 rpm for 1 minute to collect the DNA solution. Store the DNA at -20°C.

Note:

1. If downstream experiments are sensitive to pH or EDTA, elute the DNA with sterile water. The pH of the elution buffer has a significant impact on elution efficiency. If using water as the elution buffer, ensure its pH is between 7.0 and 8.5 (you can adjust the pH with NaOH). If the pH is below 7.0, elution efficiency will be low.
2. Incubating at room temperature for 5 minutes before centrifugation can increase the yield.
3. Eluting again with an additional 50-200 µL of Buffer GE or sterile water can further increase the yield.
4. To increase the final concentration of DNA, you can add the DNA elution solution obtained from step 10 back onto the spin column membrane and repeat step 10. If the elution volume is less than 200 µL, it can increase the final DNA concentration, but it may reduce the total yield. If the DNA amount is less than 1 µg, it is recommended to elute with 50 µL of Buffer GE or sterile water.
5. Since DNA stored in water is susceptible to acid hydrolysis, it is recommended to elute with Buffer GE and store the DNA at -20°C for long-term preservation.

– The End –