For research use only Version Number：2.0-1809

Animal Tissue DNA Isolation Kit

For genomic DNA purification from cultured cells and animal tissues

Kit content	DE-05011	DE-05012	DE-05013
	50T	100T	250T
Buffer L1	20 mL	40 mL	100 mL
Buffer L2*	20 mL	40 mL	100 ml
Buffer PW*	25 mL	50 mL	125 mL
Buffer WB	25 mL	50 mL	125 mL
Buffer EB	10 mL	20 mL	50 mL
Foregene Protease	1.25 mL	1.25 mL× 2	6.25 mL
DNA-Only Column	50	100	250
manual	1	1	1

*: Buffer L2 and Buffer PW contain irritating desalted salt. Please wear gloves and take relevant protective measures when operating.

Introduction

This kit uses DNA-Only Column that can specifically bind to DNA, a brand-new Foregene Protease and unique buffer system, which can extract high-quality genomic DNA from various cultured cells and animal tissues within 30-50 minutes.

The DNA-Only silica gel membrane used in the spin column is a unique new material of the company, which can efficiently and specifically adsorb DNA, which can remove RNA, impurity proteins, ions and other organic compounds in cells to the greatest extent. The extracted genomic DNA fragments are large, high in purity, and stable and reliable in quality.

Storage and Stability

This kit can be stored for 12 months under dry conditions at room temperature (15-25℃) ; if it needs to be stored for a longer period of time, it can be stored at 2-8 ℃ . Foregene Protease has a unique formula, which is active when stored at room temperature for a long time (3 months) ; its activity and stability will be better when stored at 4 ℃ ,so it is recommended to store it at 4℃ , remember not to store at -20℃ .

Warnings and Precautions

- The sample should avoid repeated freezing and thawing, otherwise the extracted DNA fragments will be smaller and the extraction volume will also decrease.
- Before use, carefully check whether there is precipitation in Buffer L1, Buffer L2 and Buffer PW. If there is precipitation, please dissolve it at 37°C and mix well before use.
- Before using the kit, be sure to check whether Buffer WB has been added with absolute ethanol according to the instructions. Add 60 mL of absolute ethanol (DE-050111), 120 mL of absolute ethanol (DE-05012), and 300 mL of absolute ethanol (DE-05013) to Buffer WB before use.
- Elution volume: Buffer EB should not be less than 100μL, otherwise it will affect the DNA yield.
- All experimental steps are carried out at room temperature (15-25℃).

Procedure

Please add absolute ethanol to Buffer WB before use. Please refer to the label on the bottle for the added volume.

A、Genomic DNA operation steps of cultured cells

1. Scrape the cells (≤5×10^6) from the culture dish with a cell scraper and collect them in a 1.5 ml clean centrifuge tube.
2. Centrifuge at 1,000 × g for 5 min , discard the cell culture medium (a pipette can be used to remove the culture medium, so as not to affect subsequent operations) , add 400μL Buffer L1 , and resuspend the cells.
3. Add 20μL Foregene Protease to the mixture, vortex to mix, place in a metal bath or water bath at 65°C for 10-20 min, vortex and mix once (or flick the bottom of the centrifuge tube with your fingers several times) To help cell enzymolysis.

   Note: The vortexing time should not be too long, just 2 seconds each time . Long-term violent vortexing will cause the genomic DNA to break into small fragments.
4. Enzyme solution is completed, add 400μL Buffer L2, at this time there will be up and down layering, invert and mix until the layering disappears, and place in a metal bath or water bath at 65°C for 10 min.
5. Centrifuge at 12,000 rpm (~13,400 ×g) for 5-10 min.

6. Transfer the supernatant to the DNA-Only Column with a pipette, and do not aspirate the precipitate.

   Note: If there are tiny precipitates in the aspirated supernatant, transfer it to a new centrifuge tube and centrifuge again, take the supernatant, and add it to the spin column.

7. Centrifuge at 12,000 rpm (~13,400 × g) for 1 min, and discard the waste liquid in the collection tube.
8. Add 500μL Buffer PW to the spin column, centrifuge at 12,000 rpm (~13,400 ×g) for 1 min , and discard the waste liquid in the collection tube.
9. Add 700μL Buffer WB to the spin column, centrifuge at 12,000 rpm (~13,400 × g) for 1 min , and discard the waste liquid in the collection tube.
10. Repeat step 9 once.
11. Put the spin column back into the collection tube and leave it out at 12,000 rpm (~13,400 ×g) for 2 minutes to remove the remaining Buffer WB from the spin column.
12. Move the spin column to a new 1.5 mL centrifuge tube, and drop 100μL of Buffer EB preheated at 65°C into the center of the membrane (do not add the eluent to the pressure ring, otherwise it will lose a lot Volume of eluent), placed at room temperature for 5 minutes, 12,000 rpm (~13,400 ×g) for 1 minute . Add 100μL of preheated Buffer EB to the center of the membrane again , and centrifuge at 12,000 rpm (~13,400 × g) for 1 min . The two collected eluates were combined.

    Note: If you want to increase the concentration of DNA, you can add the solution from the first centrifugation back to the spin column and centrifuge at 12,000 rpm (~13,400×g) for 1 min.

B. Genomic DNA manipulation steps of animal tissues

1. Weigh 10-50 mg of fresh or frozen animal tissues and cut them as much as possible to facilitate subsequent enzymatic hydrolysis.

   Note: The enzyme content in the liver is high. You can cut it into a pre-cooled mortar, add liquid nitrogen to grind it, and quickly transfer it to a clean centrifuge tube.

2. Was Add 400μL Buffer L1 and 20μL Foregene Protease to the centrifuge tube, vortex to mix, and place in a metal bath or water bath at 65°C for about 30 min. Vortex and mix every 10 min between them (or use your fingers to vigorously). Flick the bottom of the centrifuge tube several times) to help the animal tissue enzymolysis.

   Note: The vortexing time should not be too long, just 2 seconds each time . Long-term violent vortexing will cause the genomic DNA to break into small fragments.

3. After the enzymolysis is completed, add 400μL Buffer L2. At this time, the upper and lower layers will appear, Invert and mix until the layer disappears. Place in a metal bath or water bath at 65°C for 10 minutes.
4. Centrifuge at 12,000 rpm (~13,400 ×g) for 5-10 min.
5. Transfer the supernatant to the DNA-Only Column with a pipette, taking care not to suck the precipitate as much as possible.

   Note: If there are tiny precipitates in the aspirated supernatant, transfer it to a new centrifuge tube and centrifuge again, take the supernatant, and add it to the spin column.

6. Centrifuge at 12,000 rpm (~13,400 × g) for 1 min, and discard the waste liquid in the collection tube.
7. Add 500μL Buffer PW to the spin column, centrifuge at 12,000 rpm (~13,400 ×g) for 1 min , and discard the waste liquid in the collection tube.
8. Add 700μL Buffer WB to the spin column, centrifuge at 12,000 rpm (~13,400 × g) for 1 min , and discard the waste liquid in the collection tube.
9. Repeat step 8 once.
10. Put the spin column back into the collection tube and leave it out at 12,000 rpm (~13,400 ×g) for 2 minutes to remove the remaining Buffer WB from the spin column.
11. The Move the spin column to a new 1.5 mL centrifuge tube, and drop 100μL of Buffer EB preheated at 65°C into the center of the membrane (do not add the eluent to the pressure ring, otherwise it will lose a lot Volume of eluent), placed at room temperature for 5 minutes, 12,000 rpm (~13,400 ×g) for 1 minute . Add 100μL of preheated Buffer EB to the center of the membrane again, and centrifuge at 12,000 rpm (~13,400 × g) for 1 min. The two collected eluates were combined.

Note: If you want to increase the concentration of DNA, you can add the solution from the first centrifugation back to the spin column and centrifuge at 12,000 rpm (~13,400×g) for 1 min.