

## Animal tissues/cells Genomic DNA Extraction Kit

Cat No.: EXD0204

Package: 50T/100T

**Storage:** Store in dry and temperate condition (15°C-25°C), re-test period for 12 months. Storage at 2°C-8°C for longer. RNase A and Proteinase K will be shipped as accessories, please store at -20°C after receiving them.

### Kit Contents:

Component	50T	100T
RNase A	1ml	1ml×2
Protease K	1ml	1ml×2
Solution A	10ml	20ml
Solution B	10ml	20ml
Washing Buffer	15ml	15ml×2
Elution Buffer	10ml	20ml
Adsorption column	50 units	100 units
Collection tube	50 units	100 units

### Product Description

The kit is based on a centrifugal adsorption column and unique buffer system that specifically binds DNA and a unique buffer system to extract the genomic DNA from tissues and cells. The silica matrix material used in the centrifugal adsorption column is a new type of material which can bind DNA specifically, and can remove impurity proteins and other organic compounds in cells to the utmost extent. Extracted genomic DNA fragments with large, high purity, stable and reliable quality. Extracted genomic DNA can be used in a variety of routine operations, including enzyme digestion, PCR, library construction, Southern Blotting etc.

### Protocol

Note: Please add Absolute Ethanol to the Washing Buffer before use. Please refer to the label on the bottle for the added volume. Unless otherwise specified, all centrifugation steps are centrifuged at room temperature using a benchtop centrifuge.

- Sample preparation
  - Cell:** Take  $1 \times 10^6$ - $1 \times 10^7$  suspension cultured cells, centrifuge at 12000rpm for 1min. Collect cells and remove supernatant. Add 200 $\mu$ l solution A, mix thoroughly.
  - Tissue:** The tissue should not be too large, generally not more than 25mg. Homogenize with a homogenizer, preferably ground to powder form with liquid nitrogen, and fully suspend with pre-cooled PBS or sterile water. Centrifuge at 12000rpm for 1min. Collect cells and remove supernatant. Add 200 $\mu$ l solution A, mix thoroughly.
- Add 20 $\mu$ l (10mg/ml) RNase A to the suspension, incubate at 55°C for 15min.

3. Add 20 $\mu$ l (10mg/ml) Protease K, invert the tube until mix thoroughly. Digest in water bath at 55°C. The cells digested time is shorter than the tissue digested time, generally takes 1-3 hours. Mouse tails need to be digested overnight. During digestion, the centrifuge tube can be inverted several times until the sample is completely digested. Indicator of complete digestion: clear and viscous liquid.
4. Add 200 $\mu$ l Solution B, mix thoroughly. White precipitates may form, incubate at 75°C for 15-30 min, white precipitates will disappear. It shows that the sample does not digested thoroughly if the solution not clear, which may make the amount and purity of DNA extraction lower and block the column.
5. Add 200 $\mu$ l of Absolute Ethanol, mix thoroughly. Flocculent precipitate may also form on addition of ethanol, which will not affect the DNA extraction. Add the mixture into a adsorption column.
6. Centrifuge at 12000rpm for 1min, discard the flow-through and re-use the collection tube in the next step.
7. Add 600 $\mu$ l Washing Buffer to the adsorption column, Centrifuge at 12000rpm for 1min, discard the flow-through and re-use the collection tube in the next step.  
Note: Washing buffer must be diluted with absolute ethanol before use.
8. Repeat step 7 with another 600 $\mu$ l Washing buffer.
9. Centrifuge at 12000rpm for 2min. Incubate at room temperature or 50°C to dry the column. It is critical for removing ethanol from the column.
10. Place column into a new clean centrifuge tube. Add 50-200 $\mu$ l Elution buffer(pre-heated to 65°C) onto the column matrix, incubate at room temperature for 5min. Centrifuge at 12,000rpm for 1 min.
11. To increase DNA concentration, add the solution obtained from step 10 to the center of membrane again. Incubate at room temperature (15-25°C) for 2 min, and then centrifuge for 2 min at 12,000 rpm.

#### **Note**

1. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size and amount.
2. If the precipitation appears, dissolve at 65°C water bath please, which will not affect on using.
3. If the volume of eluted buffer is less than 50 $\mu$ L, it may affect recovery efficiency. The pH value of elution buffer will have big influence in eluting. If using distilled water, pH should be controlled at 8.0, below 7.0 will affect elution efficiency.