

## Animal tissue/cell genome DNA extraction kit

**Cat:** D1700

**Package:** 50T/100T

**Storage:** RT, Valid for 1 year (RNase A and protease K are delivered as accessories and stored at -20°C).

### Product composition:

Kit composition	50T	100T	Storage
RNase A	100μL	100μL×2	-20°C
Protease K	1mL	1mL×2	-20°C
Solution A	10mL	20mL	RT
Solution B	10mL	20mL	RT
Bleaching solution	15mL	15mL×2	RT
Eluent	10mL	20mL	RT
Adsorption column	50	100	
Collecting tube	50	100	
Specification	1	1	

### Notes:

1. Please add anhydrous ethanol to the bleach solution before use. Please refer to the label on the bottle to add the volume (each bottle needs to add 45mL anhydrous ethanol separately).
2. All centrifugation steps are performed using a table centrifuge at room temperature.

### Product description:

This kit uses a centrifugal adsorption column that specifically binds DNA and a unique buffer system to extract genomic DNA from tissues and cells. The silicon matrix material used in the centrifugal adsorption column is our company's unique new material, which can efficiently and specifically adsorb DNA, and can maximize the removal of foreign proteins and other organic compounds in the cell. The extracted genomic DNA fragments are large, high purity, stable and reliable. Genomic DNA extracted using this kit can be used for a variety of routine operations, including enzyme digestion, PCR, library construction, Southern hybridization and other experiments.

### Operation steps:

1. Sample handling:
  - (1) Cells:  $1 \times 10^6 - 1 \times 10^7$  suspension cultured cells were taken and centrifuged at 12000rpm for 1min to collect cells. The adherent cells were digested with trypsin first, then blown into cell suspension with pre-cooled PBS, then centrifuged at 12000rpm for 1min to collect cells, remove the supernatant as much as possible, add 200μL solution A, and shake until thoroughly mixed.
  - (2) Tissue: the amount of tissue should not be too large, generally not more than 25mg, can be homogenized by A homogenizer, preferably ground into powder with liquid nitrogen, and then fully suspended with pre-cooled PBS or sterile water, and then centrifuge 12000rpm for 1min to collect cells, remove the supernatant as far as possible, add 200μL solution A, and shake until thoroughly mixed.
2. Add 2μL RNase A to the suspension and place for 5min at 55°C.





3. Add 20 $\mu$ L protease K, fully invert and mix, 56 $^{\circ}$ C water bath digestion, cell digestion time is shorter, tissue digestion time is longer, generally takes 1-3 hours to complete (mouse tail needs to digest overnight). During digestion, the centrifuge tube can be reversed and mixed several times until the sample is completely digested. The indicators of complete digestion are: the liquid is clear and thick.
4. Add 200 $\mu$ L volume solution B and mix thoroughly upside down. If white precipitation appears, it can be placed at 75 $^{\circ}$ C for 15-30min, and the precipitation will disappear without affecting the subsequent experiment. If the solution does not become clear, it indicates that the sample is not thoroughly digested, which may lead to a small amount of extracted DNA and impure, and may lead to blockage of the adsorption column.
5. Add 200 $\mu$ L anhydrous ethanol, shake and mix thoroughly for 15s, and the solution becomes clear. At this time, flocculation precipitation may occur, which does not affect the extraction of DNA, and both the solution and the flocculation are added to the adsorption column.
6. Centrifuge at 12000rpm for 1min, discard the waste liquid, and put the adsorption column into the collection tube.
7. Add 600 $\mu$ L bleach solution to the adsorption column (check whether anhydrous ethanol has been added before use), centrifuge at 12000rpm for 1min, discard the waste liquid, and put the adsorption column into the collection pipe.
8. Repeat Step 7.
9. Centrifuge at 12000rpm for 2min and place the adsorption column open at room temperature or 50 $^{\circ}$ C for several minutes to remove the residual bleach solution in the adsorption column; otherwise, the ethanol in the bleach solution will affect subsequent experiments such as enzyme digestion and PCR.
10. Put the adsorption column into a clean centrifuge tube, add 50-200 $\mu$ L eluent preheated in a water bath at 65 $^{\circ}$ C to the center of the adsorption film, place at room temperature for 5min, and centrifuge at 12000rpm for 2min.
11. The eluent obtained by centrifugation can be added to the adsorption column and centrifuged at 12000rpm for 2min to obtain high quality genomic DNA.

#### Notes:

1. After the kit is unwrapped, RNase A and protease K should be stored at -20 $^{\circ}$ C.
2. The sample should avoid repeated freezing and thawing, otherwise the extracted DNA fragments will be smaller and the extracted amount will decrease.
3. If the solution in the kit precipitates, it can be re-dissolved in a 65 $^{\circ}$ C water bath before use, without affecting the effect.
4. The volume of the elution buffer is preferably no less than 50 $\mu$ L, too small volume will affect the recovery efficiency: the pH value of the eluent also has an impact on the elution efficiency, if you need to use water to make the eluent should ensure that its pH value is about 8.0 (NaOH can adjust the pH value of the water to this range), pH value below 7.0 will reduce the elution efficiency.

#### Related products:

D1010 6 $\times$ DNA Loading Buffer  
T1060 50 $\times$ TAE Buffer  
T1050 5 $\times$ TBE Buffer  
M1060 D2000 DNA Ladder  
M1400 1kb DNA Ladder  
G8142 GoldView Type II nucleic Acid Stain (5000 $\times$ )

