

Animal Tissue Genome DNA Extraction Kit (Magnetic bead method)

Cat: DM1700

Size: 50T/100T

Storage: 2-8°C (Note: RNase A will be shipped as an accessory, please store at -20°C; Magnetic bead, stored at 4°C, do not freeze)

Kit Components:

Kit Components	50T	100T	Storage
RNase A	100μL	100μL×2	-20°C
Protease K	20mg	20mg×2	2-8°C
Dissolved enzyme liquid	1mL	1mL×2	RT
Solution A	15mL	30mL	RT
Washing Buffer	25mL	25mL×2	RT
Elution Buffer	10mL	20mL	RT
Magnetic bead	750μL	1.5mL	2-8°C, do not freeze

Note: Please add absolute ethanol to the washing buffer before use. Please refer to the label on the bottle for the added volume (Each bottle needs to add 75mL absolute ethanol).

Introduction:

The kit uses magnetic bead method to extract genomic DNA from different tissues of animals. The specific combination of magnetic bead and DNA makes the extracted DNA of high purity and high concentration, and the operation time is short, and no other instruments are required. The genomic DNA extracted with this kit can be applied to a variety of routine molecular biology experiments, including enzyme digestion, PCR, sequencing, linking and transformation tests. This kit does not need to use benzene, chloroform and other toxic reagents, and is safe to operate.

Protocols:

Configuration of protease K solution: 1mL Dissolved enzyme liquid was added into 20mg protease K powder to form 20mg/mL protease K solution, and the prepared protease solution was stored at -20°C.

1. Take 23-26mg tissue and place in 1.5mL centrifuge tube, add 200μL solution A into the centrifuge tube, and grind the tissue completely with a grinding pestle.
2. Add 20μL of protease K solution into the centrifuge tube, shake and mix well, place at 65°C for 1-3h, remove and add 2μL RNase A into the centrifuge tube, blow and mix well, and put at room temperature for 20min.
3. Add 220μL absolute ethanol and 15μL magnetic bead into the centrifugal tube (**the magnetic bead need to be mixed by vortex shaking before use**), and put them at room temperature for





10min after vortex shaking and mixing, and reverse mix once every 2-3min.

4. Put the centrifugal tube on the magnetic separation device and adsorb it for 5min. After the magnetic bead gather, the liquid is sucked out.

5. Add 600 μ L washing buffer to the centrifugal tube (**please confirm whether absolute ethanol has been added before use**), swirl and mix well, immediately put on the magnetic separation device for adsorption, and then suck out the liquid after the magnetic beads collect liquid and clarify.

6. Repeat Step 5 twice.

7. Place the centrifuge tube on the magnetic separation device with the cover open for 5min to remove excess ethanol. If there is excess liquid at the bottom of the centrifuge tube during the standing period, use the pipette to suck it out.

8. Add 100-200 μ L elution buffer which is after 65 $^{\circ}$ C water bath to the centrifuge tube, mix well with a pipette gun, put at room temperature for 5min, and then place on the magnetic separation device for adsorption. After the liquid collected by the magnetic beads was clarified (If the absorbed liquid appears cloudy, it can be centrifuged), The liquid is the extracted genomic DNA, and the extracted DNA products should be stored at -20 $^{\circ}$ C.

Notes:

1. The magnetic bead is store in the refrigerator at 2-8 $^{\circ}$ C. Operations such as freezing, drying and centrifugation will cause agglomeration of magnetic beads, which is not easy to be resuspended and dispersed, and affect the chemical activity of functional groups on the surface of magnetic beads.

2. Genomic DNA is extracted from fresh animal tissue whenever possible.

3. When the genomic DNA of the spleen is extracted, the eluent will become viscous after being added to the warm bath. The volume of eluent can be appropriately increased (for example, 100 μ L eluent can be added to the centrifuge tube) for centrifugation.

4. If the volume of elution buffer is less than 100 μ L, it may affect recovery efficiency. The pH value of elution buffer has influence in eluting too. If using water as elution buffer, pH should be controlled at 8.0 (adjusted by NaOH), pH below 7.0 will affect elution efficiency. The extracted DNA should be at -20 $^{\circ}$ C to prevent degradation.

5. DNA concentration and purity detection: The extracted DNA fragments can be detected for concentration and purity by agarose gel electrophoresis and UV spectrophotometer. DNA should have a significant absorption peak in OD260, OD260=1 is equal to 50 μ g/mL double-stranded DNA, 40 μ g/mL single-stranded DNA. OD260/OD280 should be 1.7-1.9, the value will be lower if using distilled water in eluting, because the pH and ions will affect the light absorption value, but do not show the purity is low.

