Spin Tissue DNA Mini Kit

Cat.No.: HYC281

Product Description

This kit is designed for total DNA extraction from 1–20 mg animal tissue or cultured cell samples with a maximum input of 5 × 10^6 cells. Adopting silica-column purification technology, this workflow eliminates toxic phenol-chloroform extraction and time-consuming ethanol precipitation procedures. The entire extraction process only takes 30–60 minutes. Purified DNA can be directly applied to PCR, Southern Blot, viral DNA detection and other downstream assays.

Components

Components	50 T	250 T
gDNA Mini Columns	50	250
2mL Collection Tubes	50	250
Buffer ATL	20 mL	80 mL
Buffer DL	20 mL	80 mL
Buffer DW1*	13 mL	66 mL
Buffer DW2*	20 mL	50 mL
RNase A	10 mg	45 mg
Proteinase K	24 mg	120 mg
Protease Dissolve Buffer	5 mL	15 mL
Buffer AE	15 mL	60 mL

Storage

This kit can be stored at ambient temperature (15–25 °C) for 18 months.Proteinase K and RNase A can be shipped and stored at room temperature. For long-term storage (over 6 months), store at −20 to 8 °C. Reconstituted Proteinase K and RNase A must be preserved at −20~8 °C.

Instructions

Preparations

1. Preparation of Proteinase K solution (20 mg/mL): Add an appropriate amount of Protease Dissolve Buffer to dry Proteinase K powder to a final concentration of 20 mg/mL. Invert gently to fully dissolve the powder. The reconstituted Proteinase K shall be stored at −20~8 °C.
2. Preparation of RNase A solution (15 mg/mL): Add an appropriate amount of Protease Dissolve Buffer to dry RNase A powder to a final concentration of 15 mg/mL. Invert gently to fully dissolve the powder. The reconstituted RNase A shall be stored at −20~8 °C.
3. Prior to first use, dilute Buffer GW1 with absolute ethanol as instructed on the bottle label.
4. Prior to first use, dilute Buffer GW2 with absolute ethanol as instructed on the bottle label.

Procedure

A. Lysis and Digestion of Animal Tissue (1–20 mg)

1. Cut 1–30 mg tissue (≤10 mg for liver, lung or spleen) into the smallest possible fragments and transfer into a 1.5 mL centrifuge tube. Add 230 µL Buffer ATL and 20 µL Proteinase K, then incubate with shaking at 55 °C for 0.5–3 hours or overnight until fully digested; invert several times during incubation or keep shaking continuously.

   Notes:

   1) Optimal DNA yield and purity depend on appropriate tissue input. Excessive starting material reduces DNA yield and purity. DNA-rich tissues such as spleen, liver and kidney are limited to a maximum of 10 mg; muscle and skin tissue can be processed up to 30 mg. For low-DNA tissues, the input may be increased to 20–50 mg; proportionally scale up Buffer ATL, Buffer DL and absolute ethanol, and load the mixture onto the column in multiple aliquots at Step 6.

   2) Minced tissue shortens digestion time. Pre-treatment with liquid nitrogen grinding, mechanical homogenizer, glass homogenizer or bead mill further accelerates digestion. Digestion duration varies by tissue type and homogenization efficiency: most tissues require 0.5–3 hours, mouse tail tissue requires 6–8 hours, and overnight digestion causes no adverse effects.

2. Add 10 µL RNase A to the digested lysate, invert to mix, and incubate at ambient temperature for 10–20 min.
   Note: RNA digestion time varies by sample type. RNA-abundant tissues like liver and kidney require extended incubation of 20–30 min.
3. Add 250 µL Buffer DL, vortex vigorously for 10 sec, and incubate in a 70 °C water bath for 10 min. Proceed to Step 4.
   Note: If obvious particulate impurities remain in the lysate, centrifuge at 12,000 × g for 5 min to remove undigested debris, transfer the supernatant to a new 1.5 mL tube, then proceed to Step 5.

B. Lysis and Digestion of Cultured Cells (max. 5 × 10^6 cells)

1. Count cells, pellet by centrifugation at 500 × g for 10 min, discard culture medium, add 150 µL Buffer PBS and vortex to fully resuspend cells.
2. Add 100 µL Buffer ATL and 10 µL RNase A, mix well, and incubate for 10–15 min to digest RNA.
3. Add 250 µL Buffer DL, vortex at high speed for 10 sec, incubate at 70 °C for 10 min, then proceed to Step 4.

C. Liquid Specimens (anticoagulated blood, hemolytic fluid, cell suspension, etc.)

1. Add 20 µL Proteinase K and 250 µL whole blood or body fluid sample into a 1.5 mL centrifuge tube, mix thoroughly.
2. Add 250 µL Buffer DL to the sample and vortex for 10 sec.
3. Incubate with shaking at 1200–1400 rpm and 70 °C for 10 min, then proceed to the column purification step.

Column Purification

4. Add 250 µL absolute ethanol to the digested lysate and vortex for 10 sec.
   Note: Precipitation may form after ethanol addition for liver or spleen samples, which is normal. Pipette up and down 5–10 times to break up precipitates completely.
5. Fit a gDNA Mini Column onto a 2 mL collection tube, load the entire mixture (including precipitates) onto the column, and centrifuge at 12,000 × g for 1 min.

Note: If column clogging occurs, centrifuge at 14,000 × g for 3–5 min. Load in separate portions if the mixture volume exceeds 750 µL.

6. Discard flow-through and reinstall the column on the collection tube. Add 500 µL Buffer GW1 to the column, centrifuge at 12,000 × g for 1 min.
7. Discard flow-through and reinstall the column on the collection tube. Add 500 µL Buffer GW2 to the column, centrifuge at 12,000 × g for 1 min.
8. Discard flow-through and place the column back into the collection tube. Add 300 µL ethanol-diluted Buffer GW2 to the column, centrifuge at 12,000 × g for 2 min.
   Note: Do not invert or tilt the column during removal; avoid touching the column bottom with waste liquid in the collection tube. If contamination occurs, discard waste liquid, reinsert the column and re-centrifuge to drain residual liquid.
9. Transfer the column to a new 1.5 mL centrifuge tube. Pipette 50–100 µL Buffer AE preheated to 70 °C directly onto the center of the silica membrane, incubate for 3 min, then centrifuge at 12,000 × g for 1 min.
10. Reload the collected eluate, or add another 50–100 µL preheated (70 °C) Buffer AE onto the membrane center, incubate for 3 min, and centrifuge at 12,000 × g for 1 min. Discard the gDNA binding column. Store purified DNA at 2–8 °C for short-term storage or −20 °C for long-term preservation.