Spin Blood DNA Mini Kit

Cat.No.: HYC222

Product Description

This kit allows rapid isolation of total DNA from less than 250 µL anticoagulated whole blood, serum, plasma, milk, saliva and other liquid samples.

It performs direct lysis and digestion on whole blood or liquid matrices. Purified DNA includes genomic DNA, mitochondrial DNA, viral DNA (e.g., Hepatitis B virus), and DNA from other parasitic microbes. The recovered DNA is directly compatible with PCR, Southern Blot, viral DNA detection and other downstream experiments.

Components

Components	50 T	250 T
gDNA Mini Columns	50	250
2mL Collection Tubes	100	5×100
Buffer AL	15 mL	90 mL
Buffer DW1	30 mL	150 mL
Buffer DW2*	20 mL	2×50 mL
Proteinase K	30 mg	140 mg
Protease Dissolve Buffer	1.8 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 can be shipped and stored at room temperature; for long-term storage (> 6 months), store at −20~8 °C.

Instructions

Preparations

1. Absolute Ethanol
2. Preparation of Proteinase K solution (20 mg/mL): Add appropriate volume of Protease Dissolve Buffer to powder Proteinase K to reach a final concentration of 20 mg/mL. Invert the tube gently to fully dissolve the powder. Dry Proteinase K powder can be stored at ambient temperature for one year; the reconstituted Proteinase K solution should be aliquoted and stored at −20 to 8 °C.
3. Prior to first use, dilute Buffer DW2 with absolute ethanol as instructed on the bottle label.

Experimental Procedure

1. Add 25 µL Proteinase K into a 1.5–2.0 mL centrifuge tube.
2. Transfer 10–250 µL sample (anticoagulated whole blood, buffy coat, cell suspension, serum, plasma, milk, saliva or other liquid matrices) to the tube containing Proteinase K, then mix for 5 sec. If sample volume is less than 250 µL, adjust total volume to 250 µL with PBS Buffer or Buffer AE.
   a. For clotted blood: Homogenize thoroughly with mechanical or glass homogenizer to fully liquefy prior to extraction.
   b. Non-mammalian blood (avian, fish, etc.) contains nucleated erythrocytes with abundant DNA; only 5–20 µL blood can be processed per run.
   c. Cultured cells (maximum input: 5×10^6 cells): Centrifuge at 400 × g for 5 min to pellet cells, discard culture medium, resuspend pellet in 250 µL PBS Buffer by vortexing, then proceed to Step 3.
   d. Low-DNA-content samples: Up to 600 µL sample may be used; proportionally scale up volumes of Buffer AL, Proteinase K and absolute ethanol. Pass the liquid through the column three times repeatedly in Step 6.
3. Add 250 µL Buffer AL to the sample, invert the tube 3–5 times and vortex vigorously for 10 sec. Incubate at 70 °C for 10 min.

   Notes:

   1) For optimal digestion, use a thermomixer with shaking at 1000–1400 rpm. If using a water bath, vortex thoroughly first and invert twice during incubation.

2) **To completely remove RNA, add 10 µL RNase A after incubation, mix well and incubate at ambient temperature for 15 min to digest RNA.**

4. Add 250 µL absolute ethanol to the lysate and vortex for 10 sec.
5. Perform brief centrifugation to collect liquid droplets on tube walls.
6. Fit a gDNA Mini Column onto a new collection tube, load the entire mixture onto the column. Centrifuge at 13,000 × g for 1 min, discard the collection tube and flow-through.
7. Mount the column onto a new collection tube. Add 500 µL Buffer DW1 to the column, centrifuge at 13,000 × g for 1 min.
8. Discard flow-through and reinstall the column on the collection tube. Add 650 µL ethanol-diluted Buffer GW2 to the column, centrifuge at 13,000 × g for 1 min.
9. Discard flow-through and reinstall the column on the collection tube. Add another 650 µL ethanol-diluted Buffer GW2 to the column, centrifuge at 13,000 × g for 1 min.
10. Discard flow-through, place the column back into the collection tube, then centrifuge at 13,000 × g for 2 min.
11. 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 10,000 × g for 1 min.
12. Reload the eluate, or add another 50–100 µL preheated (70 °C) Buffer AE onto the membrane center, incubate for 3 min, and centrifuge at 10,000 × g for 1 min.
13. Discard the gDNA binding column. Store purified DNA at 2–8 °C for short-term use, or at −20 °C for long-term preservation.