Spin Circulating DNA Mini Kit

Cat.No.: HYC231

Product Description

This kit provides a simple and rapid solution for circulating DNA extraction from serum, plasma and other cell-free liquid samples. Circulating DNA is extracellular free DNA generated during cell apoptosis, with fragment sizes generally less than 1 kb. Adopting silica-column purification technology, this extraction procedure eliminates toxic phenol-chloroform extraction and time-consuming ethanol precipitation. The recovered circulating DNA can be directly applied to quantitative PCR, liquid/solid phase microarray analysis, hybridization, SNP detection and other downstream analyses. This reagent enables efficient capture of trace cell-free nucleic acids from up to 1 mL plasma.

Components

Components	50 T	250 T
Buffer ACL	50 mL	250 mL
Buffer DCW1 *	13 mL	66 mL
Buffer DCW2 *	20 mL	50 mL
Elution Buffer	15 mL	30 mL
Carrier RNA	120 μg	310 μg
Proteinase K	120 mg	5×120 mg
Protease Dissolve Buffer	15 mL	60 mL
Viral Mini Columns	50	250
2ml Collection Tube	100	5×500

Storage

This product can be stored at room temperature (15–25°C) for 18 months. The lyophilized Proteinase K / Carrier RNA can be shipped and stored at room temperature. Upon receipt, it is recommended to store them at -20～8°C. After reconstitution, Proteinase K / Carrier RNA should be kept at -20～8°C.

Instructions

Preparations

- Absolute ethanol
- Isopropanol
- Reconstitution of Proteinase K (20 mg/mL): Add an appropriate amount of Protease Dissolve Buffer into the vial containing Proteinase K powder to a final concentration of 20 mg/mL. Invert gently to fully dissolve the lyophilized Proteinase K. The reconstituted Proteinase K should be stored at -20 ℃. Repeated freeze-thaw cycles will compromise its activity.
- Add absolute ethanol to dilute Buffer DCW2 as indicated on the bottle label, and store at room temperature.
- Add absolute ethanol to dilute Buffer DCW1 as indicated on the bottle label, and store at room temperature.
- Reconstitution of Carrier RNA (0.2 μg/μL): Add an appropriate volume of reagent to the lyophilized Carrier RNA tube according to the table below to reach a final concentration of 0.2 μg/μL. Vortex thoroughly and incubate at room temperature for 10 minutes to ensure complete dissolution. Aliquot the reconstituted Carrier RNA and store at -20 ℃.

Components	50 T	250 T
Carrier RNA	120 μg	310 μg
Buffer AE	500 μL	1.4 mL
Proteinase K	5 μL	20 μL

Isolation and Storage of Plasma, Serum and Body Fluid

1. Take samples such as EDTA-anticoagulated blood, effusion and secretion. Centrifuge at 4 °C, 1900 × g (3000 rpm) for 10 minutes to remove somatic cells. Carefully transfer the collected plasma or body fluid into a high-speed centrifuge tube.
   Generally, about 4–5 mL of plasma can be obtained from 10 mL of whole blood.
2. Centrifuge at 4 °C, 16,000 × g for 10 minutes to eliminate cell debris, excess cellular nucleic acids attached to cell debris, as well as gDNA and RNA released from damaged blood cells.
3. Carefully transfer the supernatant to a new centrifuge tube without aspirating the pellet. Samples to be used on the same day can be stored at 2–8 °C until use. For long-term storage, keep samples at −80 °C.Frozen plasma, serum or body fluid samples shall be thawed completely at room temperature before use. If precipitate appears after thawing, centrifuge at

4 °C, 16,000 × g for 5 minutes, then carefully transfer the supernatant to a new centrifuge tube.

Experimental Procedure

1. Transfer 100 µL Proteinase K into a 5.0 mL centrifuge tube, then add 1.0 mL plasma, serum, urine, effusion or other cell-free body fluid samples. Invert the tube 3–5 times to mix thoroughly.
2. Add 1.0 mL Buffer ACL and (optional) 5 µL Carrier RNA to the sample, then invert 15–20 times for full mixing.
   Prior to use, pre-mix Carrier RNA with Buffer ACL. The recommended dosage of Carrier RNA per sample is 1 µg (5 µL). Carrier RNA improves DNA recovery but interferes with Qubit quantification. Its addition is optional based on experimental requirements.
3. Incubate in a 60 °C water bath for 30–60 min; invert the tube several times every 10–15 min during incubation.
4. Add 0.7 mL isopropanol to the sample, invert 3–5 times, vortex for 10–15 sec, then incubate on ice for 3–5 min.
5. Fit a Viral Mini Column onto a 2 mL collection tube, load 750 µL mixture onto the column, and centrifuge at 10,000 × g for 1 min.
6. Discard flow-through and reinstall the column onto the collection tube. Load another 750 µL mixture and centrifuge at 10,000 × g for 1 min. Repeat this step until the entire mixture has passed through the column.
7. Mount the column onto a new collection tube. Add 500 µL ethanol-diluted Buffer DCW1 to the column and centrifuge at 10,000 × g for 1 min.
8. Discard flow-through and reattach the column to the collection tube. Add 500 µL ethanol-diluted Buffer DCW2 to the column and centrifuge at 10,000 × g for 1 min.
9. Discard flow-through and reattach the column to the collection tube. Add another 500 µL ethanol-diluted Buffer DCW2 to the column and centrifuge at 10,000 × 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 and dry the column further at 56 °C for 10 min.

12. Pipette 40–50 µL Elution Buffer directly onto the center of the silica membrane, incubate for 2 min, then centrifuge at 13,000 × g for 1 min.
13. Reload the collected eluate onto the membrane center, incubate for another 2 min, and centrifuge at 13,000 × g for 1 min.
14. Discard the nucleic acid binding column. Store purified DNA at 2–8 °C for short-term use, or at −20 °C for long-term preservation.