Spin Circulating DNA Micro Kit

Cat.No.: HYC236

Product Description

Spin Circulating DNA Micro Kit provides a simple and rapid solution for the isolation of circulating DNA from serum, plasma and other cell-free liquid samples. Circulating DNA refers to cell-free extracellular DNA derived from cell apoptosis, with fragment sizes generally below 1 kb. Based on silica column purification technology, this kit eliminates the need for toxic phenol-chloroform extraction and time-consuming alcohol precipitation during the extraction procedure. The purified DNA can be directly applied to quantitative PCR, liquid-phase or solid-phase microarray analysis, hybridization, SNP detection and other downstream assays.
The Spin Circulating DNA Micro Kit is optimized for the capture of ultra-trace cell-free nucleic acids from 0.6 mL plasma samples.

Components

Components	50 T	250 T
Buffer ACL	40 mL	180 mL
Buffer DCW1 *	13 mL	66 mL
Buffer DCW2 *	20 mL	50 mL
Buffer AE	10 mL	30 mL
Carrier RNA	120 µg	310 µg
Proteinase K	36 mg	180 mg
Protease Dissolve Buffer	5 mL	15 mL
Viral Mini Columns	50	250
2ml Collection Tube	100	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 (96 ~ 100%)
- 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	550 μL	1.55 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. **Into a 2.0 mL centrifuge tube, add 30 µL Proteinase K and 0.6 mL plasma, serum, effusion or other liquid samples. Invert the tube 3 times to mix thoroughly.**
2. **Add 0.6 mL Buffer ACL and (optional) 5 µL Carrier RNA to the sample, then vortex for 10 seconds to mix.**
   Prior to use, pre-mix Carrier RNA with Buffer ACL. The amount of Carrier RNA per sample is 1 µg (5 µL). Carrier RNA helps improve DNA recovery but may interfere with Qubit quantification. Its addition is optional according to experimental requirements.
3. **Incubate in a 60 ℃ water bath for 30 minutes, and invert the tube several times occasionally during incubation for even mixing.**
4. **Add 0.3 mL isopropanol to the sample, vortex for 10 seconds, and incubate at room temperature for 5 minutes.**
5. **Place the Viral Mini Column into a 2 mL collection tube. Transfer half of the mixture into the column,** then centrifuge at 13,000 × g for 1 minute.
6. **Discard the filtrate and reassemble the column into the collection tube. Transfer the remaining mixture into the column,** centrifuge at 13,000 × g for 1 minute. Discard the filtrate and the collection tube.
7. **Fit the column into a new collection tube. Add 500 µL ethanol-diluted Buffer DCW1 to the column,** centrifuge at 13,000 × g for 1 minute.
8. **Discard the filtrate and reassemble the column into the collection tube. Add 500 µL ethanol-diluted Buffer DCW2 to the column,** centrifuge at 13,000 × g for 1 minute.
9. **Discard the filtrate and reassemble the column into the collection tube. Add another 500 µL ethanol-diluted Buffer DCW2 to the column,** centrifuge at 13,000 × g for 1 minute.
10. Discard the filtrate and reassemble the column into the collection tube. Centrifuge at 13,000 × g for 2 minutes for dry-spinning.
11. Transfer the column to a new 1.5 mL centrifuge tube, and dry the column further at 60 ℃ for 10 minutes.

12. **Place the column into a new 1.5 mL centrifuge tube. Add 30–50 µL Buffer AE or sterile water to the center of the column membrane.** Let stand for 3 minutes, then centrifuge at 13,000 × g for 1 minute.
13. **Transfer the eluate back to the center of the column membrane, incubate for another 3 minutes,** and centrifuge at 13,000 × g for 1 minute.
14. Discard the DNA binding column. Store the eluted DNA at 2–8 ℃ for short-term use; store at −20 ℃ for long-term preservation.

Troubleshooting

1. **Column clogging**

- Improper sample storage: Hemolysis occurs in blood samples. Reduce the sample loading volume.
- Excessive impurities in sample: Centrifuge the sample at 16,000 × g for 10 minutes to remove excess impurities.
- Decreased activity of Proteinase K: Prepare fresh Proteinase K. Store reconstituted Proteinase K at −20 ℃ after use. Do not pre-mix Proteinase K with Buffer ACL.
- Incomplete sample lysis: Insufficient mixing of sample and Buffer ACL. Repeat the extraction procedure. After adding Buffer ACL, invert the tube 3–5 times first, then vortex at maximum speed to ensure thorough homogenization of the sample with Buffer ACL.

2. **Low DNA purity**

- Inadequate column drying at 56 ℃: Complete removal of residual ethanol by thorough drying is critical for downstream enzymatic reactions.
- Excessive impurities in sample: Centrifuge the sample at 16,000 × g for 10 minutes to remove excess impurities.