Version No. FML1719

Plasma/Serum Circulating DNA Extraction Kit

(Spin Column)

Catalog No.: D107

Kit Content:

Content	D107 (50 preps)
Buffer DLT	45 ml
Buffer DW1	13 ml
Buffer DW2	15 ml
Buffer EB	15 ml
Proteinase K	1.2 ml
Carrier RNA	160 ul
FinePure Mini Spin Columns	50 pcs
2 ml Collection Tubes	50 pcs

Storage：

Plasma/Serum Circulating DNA Kit can be stored at room temperature (15-25℃) for up to 12 months. If any precipitation forms in the solution at low temperature, please incubate them at a 37℃ water bath to dissolve the precipitation. Carrier RNA is stored at -20℃.

This Kit Special for Scientific Research.

Introduction

Plasma/Serum Circulating DNA Kit is based on silica membrane technology and provides special buffer system. Genomic DNA binds to the silica membrane in the presence of high salt, while the contaminants pass through the column. After the membrane is thoroughly washed to remove any remaining contaminants, the pure DNA is eluted from the membrane with low salt buffer.

The kit is used for isolating circulating DNA from serum/plasma. Purified circulating DNA can directly serve as templates for PCR, DNA library construction, restriction enzyme digestion, hybridization, *etc.*.

Important Notes

1. Check whether crystallization or precipitation forms in the Buffer DLT for before use, If any, incubate it in a 60℃ water bath to completely dissolve the solution.
2. To improve DNA yield, the kit supplies Carrier RNA. Direct analysis of the circulating DNA by PCR is recommended.
3. Carrier RNA is dissolved in the stock solution with the concentration of 1ug/ul. Before use, please collect all the liquids to the bottom of the tube by briefly spinning. Divide it into conveniently sized aliquots, and store at -20°C. Do not freeze-thaw the aliquots of Carrier RNA more than 3 times.
4. **Anhydrous ethanol should be kept at room temperature below 25°C, that is, the temperature of anhydrous ethanol should not exceed 25°C; if this requirement is not met, please cool the anhydrous ethanol on ice.**
5. Before operation, a water bath (or other applicable constant temperature incubation device) at the required temperature should be prepared in advance.

Protocol

Ensure that absolute ethanol has been added to Buffer DW1 and Buffer DW2 according to the instructions at the first use.

1. Pipet 600 µl serum/plasma into a 2 ml microcentrifuge tube.
2. Add 20 µl Proteinase K, mix well by vortexing.
3. Add 600 µl Buffer DLT (add 3 µl Carrier RNA Stock Solution). Close the lid, and mix by inverting gently. Incubate at 56°C for 10 min, and softly shake the 2 ml microcentrifuge tube during incubation. Briefly spin the 2 ml microcentrifuge tube to remove drops from inside the lid.

Note: A white precipitate may form when Buffer DLT is added. The precipitate does not interfere with the procedure and will dissolve during the incubation at 56°C. If the precipitate will not dissolve, it indicates that the cell is not completely lysed and may reduce the yield and impurity of DNA.

4. Add 200 µl absolute ethanol (If room temperature exceeds 25°C, cool the ethanol on ice before adding to the 2 ml microcentrifuge tube), close the lid, and mix thoroughly by inverting gently. Incubate for 5 min at room temperature (15-25°C). Briefly centrifuge the 2 ml microtube to remove drops from inside the lid.
5. Carefully transfer the entire lysate from step 4 to the FinePure Mini Spin Column (in a 2 ml Collection Tube) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm for 30 sec. Discard the flowthrough. Replace the Spin Column in the collection tube.
6. Carefully open the Spin Column and add 500 µl Buffer DW1 (Ensure that ethanol has been added before use) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm for 30 sec. Discard the flowthrough and replace the Spin Column in the Collection Tube.
7. Carefully open the Spin Column and add 600 µl Buffer DW2 (Ensure ethanol has been added before use) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm for 30 sec. Discard the flowthrough and replace the Spin Column in the Collection Tube.
8. Repeat Step 7.
9. Replace the Spin Column in the Collection Tube, centrifuge at 12,000 rpm for 2 min and discard the flow-through. Incubate Spin Column in room temperature (15-25°C) for 2-5 min to dry the membrane completely.

   Note: This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
10. Place the Spin Column in a clean microcentrifuge tube. Apply 30-50 µl Buffer EB (Buffer EB needs to be in 65-70℃ water, water bath for 3- 5 minutes) to the center of the membrane. Close the lid and incubate at room temperature (15-25°C) for 1 min. Centrifuge at 12,000 rpm for 1 min.

    Note: The elution volume should not be less than 30 µl since smaller volume will affect recovery efficiency. If the eluent buffer is water, ensure that its pH value is between 7-8.5. For long-term storage of DNA, storing at -20°C is recommended.

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