TIANGEN®

Serum/Plasma Circulating DNA Kit

For isolation of circulating DNA from plasma and serum

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Serum/Plasma Circulating DNA Kit
(Spin Column)

Cat. no. 4992289

Kit Contents

Contents	4992289 50 preps
Buffer GA	15 ml
Buffer GB	15 ml
Buffer GD	13 ml
Buffer PW	15 ml
Buffer TB	15 ml
Proteinase K	1 ml
Carrier RNA	310 μg
RNase-Free ddH_2O	1 ml
RNase-Free Spin Columns CR2	50
Collection Tubes 2 ml	50
Handbook	1

Storage

Serum/Plasma Circulating DNA Kit should be kept in dry place and can be stored at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, the kit can be stored at 2-8°C. If a precipitate has formed in Buffer under 2-8°C, please place the buffer at room temperature or warm at 37°C for 10 min to dissolve the precipitate. Stock solution of Carrier RNA should be stored at -20°C.

Introduction

Serum/Plasma Circulating DNA Kit is based on silica membrane technology and provides special buffer system. Genomic DNA binds to the silicamembrane 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 isolation DNA from serum/plasma. Purified genomic DNA can directly serve as templates for PCR, restriction enzyme digestion, hybridization, etc.

Important Notes (Please read before use)

1. Reagents to be supplied by user: Ethanol (96-100%)
2. Technical Index of Serum/Plasma Circulating DNA Kit

Maximum Capacity of Spin Column CR2	700 µl
Minimal Elution Volume of Buffer TB	20 µl
Volume of Serum/Plasma	Maximum 200 µl

3. If a precipitate has formed in Buffer GA or GB, please warm them at 37°C to dissolve the precipitate.
4. Equilibrate samples to room temperature (15-25°C).
5. Ensure that ethanol (96-100%) has been added to Buffer GD and Buffer PW as indicated on the tag of bottle at the first use.
6. For high yield of DNA, the kit supplies Carrier RNA. Direct analysis of the genomic DNA by PCR is recommended, since use of Carrier RNA results in errors in OD_{260}.

Preparation of Carrier RNA Stock Solution

At the first use of Carrier RNA, add 310 µl RNase-Free ddH_2O to the tube containing 310 µg lyophilized Carrier RNA to obtain a solution of 1 µg/µl. Dissolve the Carrier RNA thoroughly, divide it into conveniently sized aliquots, and store at -20°C. Do not freeze-thaw the aliquots of Carrier RNA more than 3 times.

Protocol:

Ensure that ethanol (96-100%) has been added to Buffer GD and Buffer PW according to the instructions at the first use.

1. Pipet 100-200 µl serum/plasma into a 2 ml microcentrifuge tube (not supplied). If the sample is less than 100 µl, add Buffer GA to reach a final volume of 100 µl.
2. Add 20 µl Proteinase K, vortex thoroughly to mix.
3. Add 200 µl Buffer GB (add 1 µl Carrier RNA Stock Solution (1 µg/µl, please refer to page 2 for preparation of Carrier RNA Stock Solution) to Buffer GB). Close the lid, and mix by inverting gently. Incubate at 56°C for 10 min, and shake the 2 ml microcentrifuge tube during that time gently. Briefly centrifuge the 2 ml microcentrifuge tube to remove drops from inside the lid.
   Note: A white precipitate may form when Buffer GB is added. The precipitate does not interfere with the procedure and will dissolve during the heat incubation at 56°C. If the precipitate will not dissolve, it indicates that the cell is not completely lysed and may result in low yield of DNA and impurity in DNA.
4. Add 200 µl ethanol (96-100%) (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 Spin Column CR2 (in a 2 ml Collection Tube) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flowthrough. Replace the Spin Column CR2 in the collection tube
6. Carefully open the Spin Column CR2 and add 500 µl Buffer GD **(Ensure ethanol has been added before use)** without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.
7. Carefully open the Spin Column CR2 and add 600 µl Buffer PW **(Ensure ethanol has been added before use)** without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.
8. Repeat step 7.
9. Replace the Spin Column CR2 in the Collection Tube, centrifuge at 12,000 rpm (~13,400 × g) for 2 min and discard the flow-through.Incubate Spin Column CR2 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 CR2 in a clean 1.5 ml microcentrifuge tube (not provided). Apply 20-50 µl Buffer TB to the center of the membrane. Close the lid and incubate at room temperature (15-25°C) for 2-5 min. Centrifuge at 12,000 rpm (~134, 00 × g) for 2 min.

Note: The elution volume should not be less than 20 µl since smaller volume will affect recovery efficiency. For high yield of DNA, the flow-through containing DNA can be added to CR2 again, incubation at room temperature (15-25°C) for 2 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min. The pH value of eluted buffer has some influence in eluting; Buffer TB or distilled water (pH 7.0-8.5) is suggested to elute plasmid DNA. For long-term storage of DNA, eluting in Buffer TB and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.