TOOLS Cell Free DNA Kit

For isolation of genomic DNA from plasma and serum

Contents

Introduction

TOOLS Cell Free DNA Kit is based on silica membrane technology and provides a special buffer system. Genomic DNA binds to the silica membrane in the presence of high salt content, whereas the contaminants pass through the column. After the membrane is thoroughly washed to remove the remaining contaminants, the pure DNA is eluted from the membrane with a low salt buffer. The kit is used for isolation of DNA from serum/plasma. Purified genomic DNA can directly serve as templates for PCR, restriction enzyme digestion, hybridization, and other such methods.

Important notes

1. Reagents to be supplied by the 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 TBB	20 μL
Volume of Serum/Plasma	Maximum 100 μL

3. If a precipitate has formed in Buffer GBA or Buffer GBB, warm the solution 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 GBD and Buffer PBW, as indicated on the tag of the bottle at the first use.
6. For a high yield of DNA, the kit supplies Carrier RNA. Direct analysis of the genomic DNA by PCR is recommended because the use of Carrier RNA results in errors in OD260.

Preparation of Carrier RNA stock solution

At the first use of Carrier RNA, add 310 μL of RNase-free ddH_2O to the tube containing 310 μg of lyophilized Carrier RNA to obtain a 1 μg/μL solution. 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 three times.

Kit Contents

Contents	DPT-BC39 (50 preps)
Buffer GBA	15 mL
Buffer GBB	15 mL
Buffer GBD	13 mL
Buffer PBW	15 mL
Buffer TBB	15 mL
Proteinase K	1 mL
Carrier RNA	310 μg
RNase-Free ddH 2 O	1 mL
Spin Columns CB3	50
Collection Tubes（2 ml）	50

Storage

TOOLS Cell Free DNA Kit can be stored in a dry place at room temperature (15−25 °C) for up to 12 months without showing any reduction in performance and quality.

Protocol

Ensure that ethanol (96%−100%) has been added to Buffer GBD (with 17 mL of ethanol added) and Buffer PBW (with 60 mL of ethanol added) before use.

1. Pipette 100−200 μL of serum/plasma into a 2-mL microcentrifuge tube (not supplied). If the sample is <100 μL, add Buffer GBA to reach a final volume of 100 μL.
2. Add 20 μL of proteinase K, and mix thoroughly on a vortex mixer.
3. Add 200 μL of Buffer GBB (add 1 μL of Carrier RNA stock solution (1 μg/μL); please refer to page 2 for the preparation of Carrier RNA stock solution) to Buffer GBB. Close the lid, and mix by inverting gently. Incubate at 56 °C for 10 min while gently shaking the 2-mL microcentrifuge tube. Briefly centrifuge the 2 mL microcentrifuge tube to remove drops from inside the lid.

Note: A white precipitate may form when Buffer GBB 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 of 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 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 s. Discard the flow-through. Replace Spin Column CR2 in the Collection Tube.
6. Carefully open Spin Column CR2 and add 500 µL of Buffer GBD (ensure that ethanol has been added before use) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 s. Discard the flow-through, and replace the Spin Column CR2 in the Collection Tube.
7. Carefully open Spin Column CR2 and add 600 µL of Buffer PBW (ensure that ethanol has been added before use) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 s. 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 at room temperature (15−25 °C) for 2−5 min to dry the membrane completely.

Note: This step is necessary, because 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 of Buffer TBB 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 (~134,000 ×g) for 2 min.

Note: The elution volume should not be <20 µL because a smaller volume will affect recovery efficiency. For a high yield of DNA, the flow-through containing DNA can be added to CR2 again, followed by incubation at room temperature (15–25 °C) for 2 min. Centrifuge at 12,000 rpm (~13,400 ×g) for 2 min. The pH of eluted buffer can influence elution; Buffer TBB or distilled water (pH 7.0–8.5) is suggested to elute plasmid DNA. For long-term storage of DNA, eluting in Buffer TBB and storing at −20 °C is recommended because DNA stored in water may undergo acid hydrolysis.