# Foregene Cell-free DNA Isolation Kit

## USER GUIDE

## Isolation of cfDNA from plasma samples

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Manual Version A.0

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Foregene Co., Ltd| Building D2, No. 18, North Bayi Road, Wenjiang, Chengdu,Sichuan,P.R.china

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# Product information

**IMPORTANT!** Before using this product, please read and understand the "Safety Information" section in the appendix .

## ■ Product description

The Foregene Cell-free DNA Isolation Kit is designed for isolating circulating cell-free DNA (cfDNA) from human plasma, serum, body fluid, or urine samples. The kit can quickly and conveniently isolate cfDNA in three steps: lysis/binding, washing and elution. It uses specially designed silica-coated superparamagnetic beads with a unique lysis/binding system to recover circulating nucleic acids by hydrogen bonding and electrostatic forces, without adsorbing proteins and other impurities. It is suitable for high-throughput automated extraction work-stations in preparation for any downstream analysis using PCR or NGS.

## ■ Kit content and storage

Table 1 Foregene Cell-free DNA Isolation Kit

| Contents | Reagent | Amount (25 T) | Amount (50 T) | Amount (100 T) | Storage |
|---|---|---|---|---|---|
| Box1 | Buffer AL | 42 mLx1 bottle | 89 mLx1 bottle | 189 mLx1 bottle | 10-30°C |
| | Buffer AW1 | 38 mLx1 bottle | 83 mLx1 bottle | 165 mLx1 bottle | 10-30°C |
| | Buffer AW2 | 15 mLx1 bottle | 33 mLx1 bottle | 66 mLx1 bottle | 10-30°C |
| | Elution | 3 mLx1 bottle | 6 mLx1 bottle | 12 mLx1 bottle | 10-30°C |
| Box2 | Magnetic Beads A | 3.2 mLx1 bottle | 6.5 mLx1 bottle | 13 mLx1 bottle | 2-8°C |
| | Proteinase K | 5 mLx1 bottle | 10.5 mLx1 bottle | 21 mLx1 bottle | 2-8°C |

Notes:

[1] Do not mix components from different batches of kits.

[2] Buffer AL may form precipitates, which does not affect its function. If precipitation is visible, please place the reagent bottle in a 56°C water bath for 10-20 min until the precipitate dissolves. Then mix thoroughly before use.

[3] Do not freeze the Magnetic Beads A.

## Required equipment and materials not supplied

Table 2 Materials required for cfDNA isolation

| Item | Note |
| --- | --- |
| Equipment | |
| Bench-top centrifuge | Rotation speed not lower than 10,000 rpm/min |
| Handheld Mini Centrifuge | / |
| Vortex | / |
| Thermostatic metal bath | / |
| Magnetic Separation Device | 1.5 mL, 5mL |
| Micro-Pipettes | 1 mL, 200 μL, 20 μL, 10 μL |
| Reagents | |
| Absolute Ethanol (>80%) | Analytical Grade |
| Isopropanol | Analytical Grade |
| Consumables | |
| Centrifuge tubes | 5 mL, 1.5 mL |
| Aerosol-resistant pipette tips | 1 mL, 200 μL, 20 μL, 10 μL |

# Methods

## Important points before starting

- Avoid repeatedly freezing and thawing samples, which may result in low DNA quality and yield.
- All reagents and samples need to equilibrate to room temperature (10°C ~30°C) before use.
- Please use the recommended consumables for automated or manual operations.
- Please read the manual carefully before the experiment.

## Things to do before starting

- Prepare the buffers according to Table 3. Add isopropanol to Buffer AL and add absolute ethanol to Buffer AW1 and Buffer AW2. Mix well, label and store at room temperature.
- If precipitates are visible in Buffer AL or Buffer AW1, dissolve by placing bottle in a 56 °C water bath. Shake well to mix before use.
- Centrifuge pre-cooling to 4°C.
- Remove the magnetic bead from 2-8℃ and re-warm it at room temperature for 30 min, vortex it well before use.
- Sample pretreatment: Plasma samples were centrifuged at 4°C for 10 min at 16000 g, and the supernatant was taken from 0.5-4 mL.
- Prepare the necessary reagents and consumables according to Table 4. The materials needed for a 0.5-4 mL sample is shown. Volumes can be adjusted accordingly.

Table 3 Buffer preparation: addition of isopropanol / ethanol

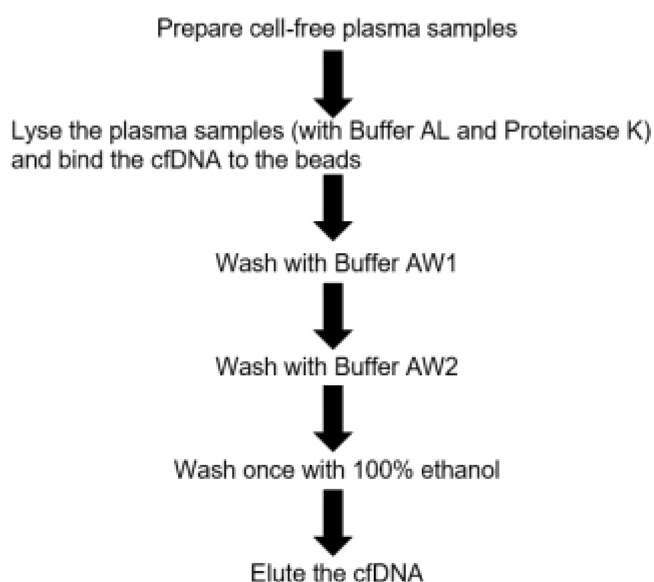
| Reagent | 2 mL×25 T | 2 mL×50 T | 2 mL×100 T |
|---|---|---|---|
| Add isopropanol to Buffer AL | 8 mL | 17 mL | 36 mL |
| Add ethanol to Buffer AW1 | 38 mL | 83 mL | 165 mL |
| Add ethanol to Buffer AW2 | 60 mL | 132 mL | 264 mL |

Table 4 Consumables and Reagents for 0.5-4 mL Sample

| Sample Volume | 0.5 mL | 1 mL | 2 mL | 4 mL |
|---|---|---|---|---|
| **Consumables** | **Volume** | **Volume** | **Volume** | **Volume** |
| Centrifuge Tube | 1.5 mL | 5 mL | 5 mL | 10 mL |
| **Reagents** | **Volume** | **Volume** | **Volume** | **Volume** |
| Proteinase K | 50 μL | 100 μL | 200 μL | 400 μL |
| Buffer AL | 0.5 mL | 1 mL | 2 mL | 4 mL |
| Magnetic Beads A | 32 μL | 62.5 μL | 125 μL | 250 μL |

| Buffer AW2 | 1.5 mL | 1.5 – 3 mL | 3 mL | 3 mL |
|---|---|---|---|---|
| Buffer AW2 | 1.5 mL | 1.5 – 3 mL | 3 mL | 3 mL |

## ■ Procedure




1. Pipet 2 mL serum or plasma into a 5 mL centrifuge tube (not provided).

2. Add 200 uL Proteinase K, 2 mL Buffer AL, 125 uL Magnetic Beads A to the 5 mL tube in order. Close the cap and mix by pulse-vortexing for 30 s.

   **Note：** Mix Magnetic Beads A thoroughly before use

3. Incubate at room temperature for 10 min. Vortex every 5 minutes for 5s.

4. Place the tube on a 5 mL magnetic stand for 3 min. After the liquid clears, carefully discard the supernatant .

. **Note：** 1.5 minutes after placing the tube on the magnetic stand, invert the stand once to collect any remaining beads on the lid.

. **Note：** Discard as much of the supernatant as possible.

5. Remove the centrifuge tube from the magnetic stand. Add 3 mL Buffer AW1 and thoroughly mix for 10s.

6. Place the centrifuge tube on the magnetic stand for 2 min. After the liquid is completely clear, carefully discard the supernatant.

- **Note：** 1 minute after placing the tube on the magnetic stand, invert the stand once to collect any remaining beads on the lid.

7. Remove the centrifuge tube from the magnetic stand. Add 3 mL Buffer AW2 and mix well for 10s.

8. Place the centrifuge tube on the magnetic stand for 2 min. After the liquid is completely clear, carefully

discard the supernatant.

- **Note：** 1 minute after placing the tube on the magnetic stand, invert the stand once to collect any remaining beads on the lid.

9. Remove the centrifuge tube from the magnetic stand. Add 3 mL Absolute Ethanol and mix well for 10s.

10. Place the centrifuge tube on the magnetic stand for 2 min. After the liquid is completely clear, carefully discard 2 mL of the supernatant.

- **Note：** 1 minute after placing the tube on the magnetic stand, invert the stand once to collect any remaining beads on the lid.

11. Remove the centrifuge tube from the magnetic stand. Mix the remaining 1 mL supernatant thoroughly and transfer to a new 1.5 mL microcentrifuge tube (not provided).

12. Place the 1.5mL centrifuge tube on a 1.5 mL magnetic stand for 2 min. After the liquid is completely clear, carefully discard the supernatant.

- **Note：** Discard as much of the supernatant as possible.

13. Remove the centrifuge tube from the magnetic stand and let dry at 56°C for 6-10min in a thermostatic heat block, until the surface of the magnetic beads becomes matte then slightly dry .

14. Remove the centrifuge tube from the heat block. Add 20- 100 μL Elution, mix by pulse-vortexing 30s.

15. Incubate at room temperature for 5 min.

16. Place the centrifuge tube on the magnetic stand for 2 min. After the liquid is completely clear, carefully transfer the supernatant containing DNA to a new 1.5 mL centrifuge tube. Label and store at 4°C for up to 24 hours or at –20°C for long-term storage.

# Appendix A

## ■ Recommendation for plasma separation and storage

To isolate circulating, cell-free nucleic acids from whole blood samples, we recommend using the following protocol which includes a high g-force centrifugation step to remove formed elements and thereby reducing the amount of cellular or genomic DNA in the sample.

1. Place a 10 mL primary blood collection tubes containing EDTA as anti-coagulant) containing EDTA-anticoagulated whole blood in a centrifuge with swing-out rotor and appropriate buckets.

2. Centrifuge whole blood samples for 10 min at 1,800 x g at room temperature (20-25°C) setting.

3. Carefully aspirate plasma supernatant without disturbing the buffy coat layer and transfer into a new 5 mL centrifuge tube. About 4–5 mL of plasma can be obtained from one 10 mL primary blood tube.

   3.1 If not immediately used: please store plasma in -80°C refrigerator. And Step 3.2 is required before use.

   3.2 For immediate use: Centrifuge plasma samples for 10 min at 16,000 x g and 4°C to remove additional cellular nucleic acids contaminants attached to cell debris.

- **Note:** Plasma can be used for circulating nucleic acid extraction at this stage. However, an additional high-speed centrifugation step may further remove any remaining cellular debris and contaminating circulating nucleic acids from gDNA and RNA derived from damaged blood cells.

4. Carefully remove supernatant to a new 1.5 mL microcentrifuge tube with a pipette without disturbing the pellet.

5. If plasma is used for nucleic acid extraction on the same day, store at 2–8°C. For long term storage, keep plasma frozen at –80°C. Before using the plasma for circulating nucleic acid extraction, place on ice until thawed.

## ■ Troubleshooting

| Observation | Possible cause | Recommended action |
|---|---|---|
| Lower yield than expected | Magnetic Beads were not properly stored | 1. Do not freeze the Magnetic Beads, store at 2–8°C.<br>2. Allow the Magnetic beads to equilibrate to room temperature before use. |
| | An insufficient amount of Magnetic Beads was added | 1. Thoroughly vortex the tube containing the magnetic beads immediately before use.<br>2. Mix the sample evenly before adding magnetic beads. |
| | The Magnetic Beads are not optimally dried | Drying times may vary depending on the amount of beads used and the environment. |

| Observation | Possible cause | Recommended action |
|---|---|---|
| | | Overdried beads will stick to the wall of the plastics and be difficult to re- suspend. Un- der- dried beads may carry ethanol into the eluate and negatively impact downstream applications. |
| | The sample contains low levels of cfDNA | Increase the starting sample volume. |
| | The sample is repeatedly frozen and thawed | Use fresh samples or samples thawed only once for extraction. |
| | Insufficient mixing of the sam- ples with the magnetic beads during the binding step | Mix vigorously by pulse- vortexing on the highest setting for 30-60s and ensure the liq- uid is adequately mixed. |
| | Magnetic Beads loss | When aspirating and discarding unwanted supernatants, ensure sufficient time for mag- netization and be careful not to discard Mag- netic Beads. |
| | Buffer AL or Buffer AW1 con- tains precipitates | Heat the bottle in a water bath at 56°C to complete dissolution. |
| | No alcohol added to reagent | Add isopropanol to Buffer AL and absolute ethanol to Buffer AW1 and Buffer AW2 be- fore use. |
| Magnetic Bead carryover | Loose beads present in the elu- ate or inadvertently transferred | 1. Be sure to leave the tube on the mag- netic stand when removing the eluate containing the cfDNA. Try not to touch the Magnetic Beads during transfer.<br>2. If beads are carried over into the new tube, place the tube on the magnetic stand again, wait for the beads to pellet and then transfer the sample to another tube. |
| Abundance of gDNA in eluate | There are cell fragments in the sample | When EDTA or citrate is used as anticoagu- lant, it is recommended to perform plasma separation immediately after blood collection to prevent gDNA from being released into plasma. At the same time, it is suggested to remove cell debris by high- speed centrifuga- tion (16,000 × g for 10 min at 4°C) after plasma sample separation. |

| Observation | Possible cause | Recommended action |
| --- | --- | --- |
| | | |
| The extraction negatively affects any downstream protocols involving enzymatic reactions. | An excess of residual detergent or ethanol remains in the extraction | 1. Discard detergent supernatant as much as possible.<br>2. Ensure optimal drying time for Magnetic Beads. |

# Appendix B

## ■ Yield and quality measurement

- **Quantitation of cfDNA Yield:**

Total cfDNA yield: We recommend using the dsDNA HS Assay Kit (Foregene ,Cat. No. DL01BT1000) for the quantitation of total cfDNA yield.

Depending on sample volume, the dsDNA HS Assay Kit is designed to accept input DNA concentrations between 0.01 and 120 ng/μL, providing a detection range of 0.2–2400 ng in 20 μL elution volume. This assay will, however, underestimate the yield of the shorter cfDNA.

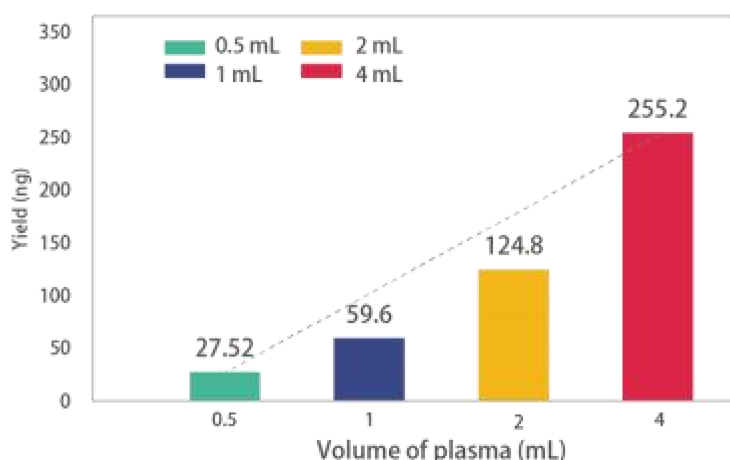



Figure 1. The recovery efficiency of cfDNA at different volumes of plasma samples . Extractions of cfDNA from 0.5 mL, 1 mL, 2 mL and 4 mL plasma samples were performed using Foregene Cell-free DNA Isolation Kit. A linear relationship can be observed between cfDNA yield and plasma sample input volume.

- **Assessment of cfDNA Quality:**

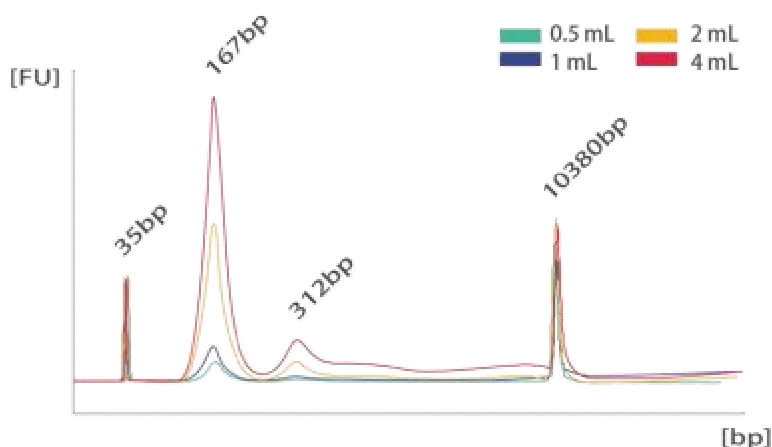


Figure 2. Agilent™ High Sensitivity Analysis on 1 μL of cfDNA isolated from 0.5 mL, 1 mL, 2 mL and 4 mL of plasma using Foregene Cell-free DNA Isolation Kit.

Through Agilent™ 2100 DNA detection, cfDNA extractions from various volumes of plasma show major peaks at about 167 bp, and demonstrate consistency in peak characteristics such as shape and intensity.

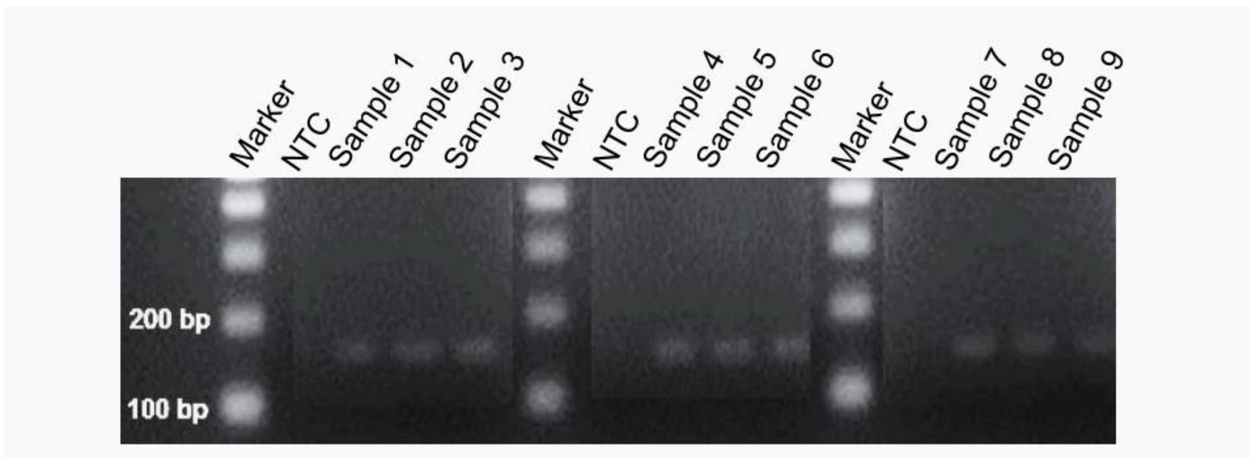



Figure 3. Gel electrophoresis image of cfDNA extractions from plasma using Foregene Cell-free DNA Isolation Kit.

Based on gel electrophoresis images of cfDNA extractions by Foregene Cell-free DNA Isolation Kit from nine plasma samples, it is shown that cfDNA fragments at roughly 167bp has been successfully isolated.

# Appendix C

## ■ Safety information

Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc.). SDSs in PDF format can be found online at https://www.foreivd.com/.

# Documentation and support

## ■ Customer and technical support

Visit www.foreivd.com for the latest service and support information.

. Telephone numbers: +86 83360257

## ■ Limited product warranty

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