# ImaBeads® Circulating Nucleic Acid Kit

For concentration and purification of free-circulating DNA, RNA, miRNA, and viral nucleic acids from human plasma, serum, urine, or other cell-free body fluids.

**Precautions**

I. Handling Requirements
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

II. Equipment and Reagents to Be Supplied by User

- Ethanol (96–100%)*
- Isopropanol
- 15 ml centrifuge tubes
- 1.5 ml microcentrifuge tubes
- Pipet tips with aerosol barrier
- Vortexer
- Water bath or heating block at 56°C

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

I. Waste Handling

- Treat waste with the country, federal, state and local regulations.

II. Important points before use

- Do not use the product if it has expired.
- Vortex magnetic Imabeads to ensure they are in suspension prior to initial use.
- Be sure and allow magnetic beads to disperse completely during the binding, wash and elution steps.
- Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

**Kit Contents**

- ICL Buffer
- ICB Buffer
- ICW1 Buffer
- ICW2 Buffer
- Elution Buffer
- ImaBeads
- Proteinase K

**Storage and Stability:**

1. This kit should be stored at room temperature.
2. Proteinase K should be stored at 4°C upon arrival.

**Description**

**ImaBeads® Circulating Nucleic Acid Kit** is designed for purification of circulating nucleic acid. Nucleic acid will be binded to magnetic beads. After washing off the contaminants, the purified DNA is eluted by low salt elution buffer. Purified nucleic acid is suitable for downstream Real-time PCR, NGS or other molecular biology applications.

**ImaBeads® Circulating Nucleic Acid Kit Test Data**

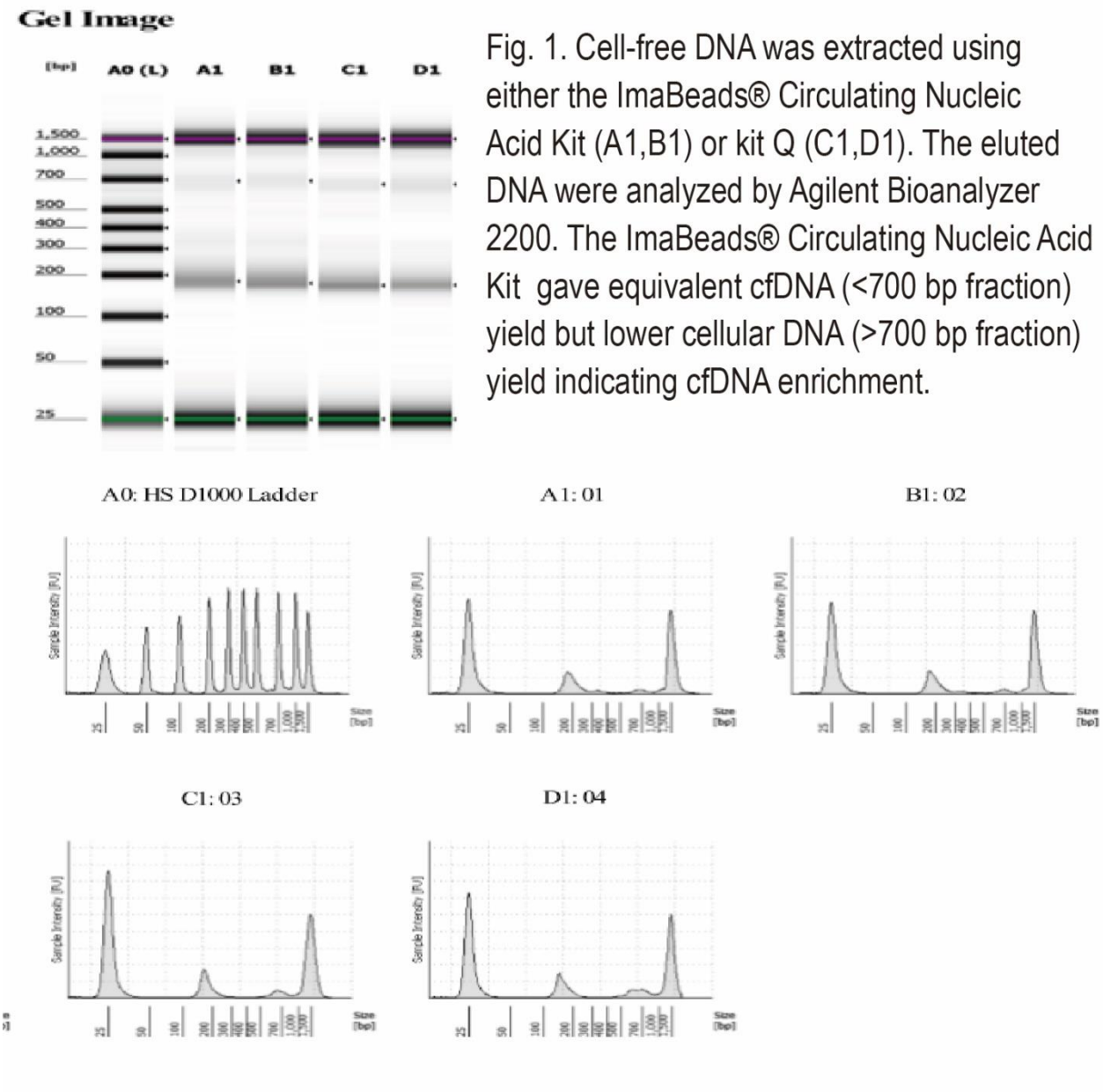


Fig. 1. Cell-free DNA was extracted using either the ImaBeads® Circulating Nucleic Acid Kit (A1,B1) or kit Q (C1,D1). The eluted DNA were analyzed by Agilent Bioanalyzer 2200. The ImaBeads® Circulating Nucleic Acid Kit gave equivalent cfDNA (<700 bp fraction) yield but lower cellular DNA (>700 bp fraction) yield indicating cfDNA enrichment.

**Preparation before using**

Add 1.1ml dd$H_2O$ to the Proteinase K tube and mix by vortexing. Store prepared Proteinase K (10 mg/ml) at 4°C.

**Protocol**

- **Sample Pretreatment**

1. Centrifuge the whole blood samples at 1900 x g for 15 minutes at 4°C by using a swing-out rotor.
2. Aspirate and transfer the plasma supernatant into 15 ml or 1.5 ml centrifuge tubes (not provided). Take care not to disturb the buffy coat and the cellular fraction.
3. Centrifuge at 16000 x g and 4°C for 10 minutes in fixed-angle rotor. Transfer the supernatant into fresh centrifuge tubes for subsequent extraction.

- **Nucleic Acid Extraction**

4. Add 0.2 ml of Proteinase K (10 mg/ml), 2 ml of ICL Buffer and 2 ml of plasma sample (from step 3) into a 15 ml centrifuge tube. Tighten the cap of the centrifuge tube to avoid leakage and vortex for 10 seconds immediately to mix the sample thoroughly.
5. Incubate at 56°C for 30 minutes.
6. Vortex the magnetic ImaBeads and add 22.5 µl of magnetic ImaBeads suspension into the sample mixture. Add 2 ml of ICB Buffer to the mixture and vortex thoroughly. Place the tube on a mixer and shake for 25 minutes to bind the cell-free DNA to the beads.
7. Place the tube on a magnetic stand for 1-2 minutes until the solution clears and aspirate the supernatant with a pipette.
8. Resuspense the beads pellet with 1000µl of ICW1 Buffer and transfer the mixture to a 1.5 ml microcentrifuge tube. Vortex the mixture for 2 minutes.
9. Place the tube on a magnetic separator for 1 minute or until the ImaBeads have pelleted. Discard the cleared supernatant with a pipette carefully.
10. Repeat step 8-9 for a second wash with ICW1 Buffer.
11. Add 1000 µl of ICW2 Buffer to the tube and mix by vortexing for 2 minutes.
12. Place the tube on a magnetic separator for 1 minute or until the ImaBeads have pelleted. Discard the cleared supernatant with a pipette carefully.
13. Repeat step 11-12 for a second wash with ICW2 Buffer.
14. Carefully remove any residual liquid. Keep the lid opened and incubate the tube on a 56°C heat plate for 15 minutes to dry the ImaBeads.
15. Add 30-100 µl Elution Buffer to the ImaBeads pellet. Close the lid and mix thoroughly by vortex for 10 seconds.
16. Incubate the tube on a 56°C heat plate for total 15 minutes and mix by votexing for 10 seconds every 3 minutes.
17. Place the tube on a magnetic separator for 1 minute or until the ImaBeads have pelleted. Transfer the cleared supernatant to a 1.5 ml RNase-free microcentrifuge tube.
18. The purified cfDNA is ready for immediate use. Store the eluate at -20°C if not used immediately.

**Troubleshooting**

| Problem | Possible Reasons/Solution |
|---|---|
| Low Yield | 1. Samples were left standing at room temperature for too long. Repeat the extraction procedure with new sample.<br>2. Low percentage ethanol used. Add absolute ethanol (see the bottle label for volume) to Buffer ICW1 and Buffer ICW2 then mix by shaking for a few seconds. Close the bottle tightly after each use to avoid ethanol evaporation.<br>3. Inefficient sample lysis. Proteninase K will lose activity for a prolong time under elevated temperature. Repeat the extraction procedure with new sample and fresh proteninase K<br>4. Buffer ICB mix incompletely. Following ICB addition, break up any precipitate as much as possible.<br>5. Sample frozen and thawed more than once. Repeated freezing and thawing will cause DNA degradation. Always use fresh sample or samples thawed only once. |