Cell-Free DNA Purification Kit

Cell-free DNA (or cfDNA) refers to all non-encapsulated double-stranded extracellular DNA fragments in the bloodstream and other bodily fluids, such as circulating tumor DNA (ctDNA), cell-free mitochondrial DNA (ccf mtDNA), and cell-free fetal DNA (cffDNA). In clinical oncology, cfDNA has emerged as a game-changing technique. The ability to detect the presence, quantity, and composition of tumor DNA using a routine, noninvasive blood sample has paved the way for a wide range of high-impact clinical applications.

Multiple cell-free DNA purification methods and numerous kits based on these methods and protocol variations exist. These various procedures and alterations result in a diverse set of cell-free DNA purification processes, which may affect cell-free DNA yield and purity. Due to the trace amount of circulating nucleic acid present in cell-free samples, scalable isolation of circulating nucleic acid from plasma, serum, and urine cfDNA recovery is challenging.

BcMag™ Cell-Free DNA Purification Kit is designed to extract cfDNA from human plasma efficiently and sequentially. The kit uses our unique proprietary magnetic beads and an optimized buffer system. It can efficiently isolate circulating cell-free DNA (cfDNA) from 100 µL -10 ml (Typical yields about 25 nanograms of cfDNA) of human plasma using Cell-Free DNA BCT collecting tubes. The procedure employs mild lysis conditions, avoiding harsh conditions such as alkaline lysis and toxic chemicals for lysing cells to maintain DNA integrity and the time-consuming cleanup of organic solvent from the sample. Purified cfDNA has the highest integrity and can be used in various downstream applications such as qPCR, ddPCR, NGS analysis, etc. When combined with an automation system, multiple samples can be isolated simultaneously. Alternatively, samples can be processed manually using a magnetic stand.

Magnetic beads have several advantages over conventional approaches for isolating cfDNA. Beads more efficiently bind the DNA than glass fiber filters, resulting in higher and more consistent yields. Furthermore, because there are no filters or vacuum manifolds employed, there is no possibility of cellular particles clogging these things during the extraction process. This clogging problem is especially problematic in protein-rich, large-volume samples like plasma, which are often utilized in cfDNA research.

Feature and advantage

- The scalable format allows for higher and lower input volumes
- Flexible design that enables both manual and automatic cfDNA isolation
- Automation-ready, phenol-free extraction
- Input volume ranges from 100µL to 10 mL of plasma
- Elution quantities range from 15 µL to 50 µL

Workflow (Fig.1)

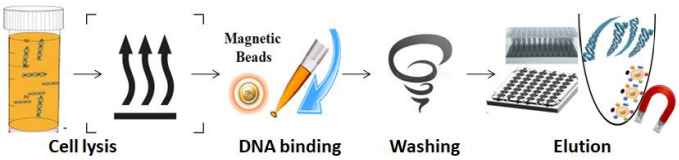

Fig.1 Workflow of cfDNA Purification Kit

1. Add lysis buffer and proteinase K to the sample to lyse the bone and incubate at 65°C.
2. Add functional magnetic beads and vortex/pipette the beads with the sample to the DNA.
3. Wash the beads.
4. Separate the beads from the sample using a magnet.
5. Elute DNA from the beads.

Products

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Components	Storage	Cat #: AC101	Cat #: AC102
BcMag™ HO-DNA Beads	4°C	1.0 ml	2 ml
10x Lysis Buffer	4°C	5 ml	10 ml
1x Elution Buffer	4°C	0.5ml	1.0 ml
Proteinase K (20mg/ml)	-20°C	15 mg	30 mg
Proteinase K Suspension Buffer	4°C	1.0 ml	2.0 ml
SDS (20%)	Room temperature	2.5 ml	5 ml
DTT (20%)	-20°C	2.5 ml	5 ml

PROTOCOL

The following protocol is an example. The protocol can be scaled up or down as needed.

Notes

- DNA yield: The yield and quality of circulating DNA are highly influenced by a blood sample, processing, storage, and plasma preparation. It is strongly advised to carry out these stages as uniformly as possible to obtain maximum reproducibility—cell-free DNA yields between 1 and 100 ng per ml of plasma. To quantify purified cfDNA, we recommend utilizing the Qubit™ dsDNA High Sensitivity Assay.
- DNA size: Varies (depends on the quality of starting material
- For long-term storage, store the extracted nucleic acids at -20°C.
- Proteinase K preparation: Provide protease K as lyophilized powder and dissolve at a 20 mg/ml concentration in Proteinase K Suspension Buffer. Divide the stock solution into small aliquots and store at -20°C. Each aliquot can be thawed and refrozen several times but should then be discarded.
- DTT solution preparation: Provide DTT as powder and dissolve at a concentration of 20% in dH_2O. It is stable for years at -20°C. Prepare in small aliquots, thaw it on ice, and use and discard. Store them in the dark (wrapped in aluminum foil) at -20°C. Do not autoclave DTT or solutions containing it. Avoid multiple freeze-thaw cycles.

A. Materials Required by the User

- 95–100% ethanol
- Isopropanol
- 65°C Incubator chamber
- Microcentrifuge tubes
- Aerosol-resistant micropipette tip
- Magnetic rack: Based on sample volume, the user can choose one of the following magnetic Racks:
  BcMag™ Rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01)
  BcMag™ Rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02)
  BcMag™ Rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03)
  BcMag™Rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-04)

B. Sample preparation

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Preparation of plasma

1. Centrifuge fresh blood sample for 10 minutes at 2,000 x g.
2. Transfer the plasma into a new tube without disturbing the sedimented cells.
3. Centrifuge the plasma samples at 16,000 × *g* for 10 minutes at 4°C.
4. Freeze plasma at -20 °C until DNA isolation.

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5. Prior to DNA isolation, thaw frozen plasma samples and centrifuge for 10 minutes at 16,000 x g at 4°C in a microcentrifuge for small plasma volumes or 30 minutes at 4,500 x g at 4°C in a tabletop centrifuge for larger plasma volumes to remove remaining cells, cell debris, and particulate matter. Make use of the supernatant to isolate DNA.

C. **Purification**

1. Prepare the plasma samples by thawing frozen plasma at room temperature. Centrifuge plasma for 5 minutes at 12000x g to remove any blood and cell debris.
2. Add the following components to a tube in the order indicated and vortexing for 1 min to mix well. Briefly spin down.

Plasma	10x Lysis buffer	Proteinase K (20mg/ml)	20% SDS[1]	DTT (20%)
0.1 ml	10 µL	15 µL	5 µL	5 µL
0.5 ml	50 µL	75 µL	25µL	25 µL
1ml	100 µL	150 µL	50µL	50 µL
2ml	200 µL	300 µL	100µL	100 µL
5ml	500µL	750µL	250 µL	250 µL
10ml	1000µL	1.5 mL	500 µL	500 µL

[1] Do not add SDS directly to the Proteinase K solution to avoid inactivation of the Proteinase K.

3. Vortexing for 1 min to mix well. Briefly spin down.
4. Incubate at 65°C for 30 min.
5. After the 30-minute incubation period, place the tubes containing the plasma sample on ice for 5 minutes to cool them to room temperature.
6. Centrifuge for 10 minutes at 16,000 x g and transfer the supernatant to a new appropriate centrifuge tube
7. Prepare the Binding Solution/Beads/Isopropanol Mix according to the following table and mix thoroughly.
8. Add the appropriate amount of beads and Isopropyl based on the following table.

Plasma	BcMag™ HO-DNA Beads**	Isopropyl alcohol
0.1 ml	5µL	82 µL
0.5 ml	15µL	410 µL
1ml	30µL	820 µL
2ml	60µL	1.64 mL
5ml	100µL	4.1mL
10ml	200µL	8.2mL

** Vigorously shake the bottle until the magnetic beads become homogeneous before dispensing.
Do not allow the beads to sit for more than 2 minutes before dispensing. Resuspend the magnetic beads every 2 minutes.

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10. Place the tube on the magnetic Rack for 1-3 minutes. Remove the supernatant while the tube remains on the Rack. Add 500µL-1000 µL of 85% Ethanol and mix by vortex for 5 minutes or pipetting 25-35 times to wash the beads. Place the tube on the magnetic Rack for 1-3 minutes and remove the supernatant completely while the tube remains on the Rack.
11. Repeat step (10) two times.
12. Remove the tube from the magnetic Rack and let the beads air dry for 10-30 minutes to evaporate the ethanol completely.
13. Add an appropriate amount of 1x Elution buffer based on the following table and mix by vortex for 5 minutes or pipetting 25-35 times to elute the DNA from the eads.

BcMag™ HO-DNA Beads**	1x Elution Buffer
5µL	10 µL
15µL	10 µL
30µL	15 µL
60µL	25 µL
100µL	50µL
200µL	75 µL

14. Place the tube on the magnetic Rack for 1-3 minutes and transfer the supernatant to a new centrifuge tube. The eluted DNA should be stored at -20°C.

Related Products

Products and Catalog Number	
Genomic DNA and RNA Purification	
One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Step Saliva Viral RNA-DNA Purification Kit, Cat. No. AR101
Cell-Free DNA Purification Kit, Cat. No AC101	Bone-Teeth DNA Purification Kit, Cat. No. AB101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ-101	Rootless Hair DNA Purification Kit, Cat. No. AD101
One-Step Bacteria DNA Purification Kit, Cat. No. AE101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Touch DNA Purification Kit, Cat. No. AS101
One-Step Fungi &Yeast DNA Purification Kit, Cat. No. AL101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101
One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101	One-Step Dandruff DNA Purification Kit, Cat. No. AAA101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	Quick mRNA Purification Kit, Cat. No. MMS101
DNA & RNA Sample Preparation	
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step DNA-RNA Removal Kit, Cat. No. CA103
One-Step RNA Removal Kit, Cat. No. AU101	One-Step DNA/RNA Cleanup Kit, Cat. No. AH101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
Quick Oligo-DNA Conjugation Kit, Cat. No. CA101	One-Step Fluorescent Labeling Cleanup Kit, Cat. No. AK101
One-Step DNA-RNA Removal Kit, Cat. No. AV101	One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101
One-Step PCR Inhibitor Removal Kit, Cat. No. AX101	Pure Miniprep Plasmid DNA Purification Kit, Cat. No. AY101

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