Cat. No. GM2016

DeepCatch™ Cell-Free Total Nucleic Acids Isolation Kit (Plasma/Serum)

For Research Use Only

Introduction

DeepCatch™ Cell-Free Total Nucleic Acids Isolation Kit is a high-performance, magnetic bead-based system optimized for the extraction of total cell-free nucleic acids from plasma and serum. Built on proprietary DeepCatch™ platform technology, the kit offers the following key advantages:

- **True Full-Spectrum cfNA Recovery & High Yield**
  Conventional isolation methods typically exhibit a marked decline in recovery for fragments below 30 nt, with severe losses below 20 nt. DeepCatch™ overcomes this limitation by delivering true full-spectrum recovery of both cfDNA and cfRNA down to 10 nt, thereby preserving the native fragment landscape. The system achieves up to ~90% recovery for ultra-short cfNA—including the challenging <20 nt fraction. This high yield capture ensures robust detection of low-abundance targets and enables biomarker discovery that conventional kits frequently miss.

- **Flexible Ultra-Short Enrichment**
  The kit provides two user-selectable enrichment modes: recovery down to 15 nt for standard ultra-short cfNA profiling (Standard Mode), or recovery down to 10 nt for discovery-oriented applications requiring maximum fragment coverage (Discovery Mode).

- **Carrier-Free Performance**
  DeepCatch™ eliminates the need for carrier nucleic acid, PEG, glycogen, LPA, or similar precipitation additives. This helps reduce non-sample background and minimizes carryover components that may interfere with downstream enzymatic reactions, including qPCR, ddPCR, ligation-based library preparation, and next-generation sequencing.

- **Multi-Omic Co-Isolation & Advanced Liquid Biopsy Applications**
  By co-isolating cfDNA and cfRNA from the same plasma or serum sample, DeepCatch™ provides a more complete cell-free nucleic acid profile while minimizing sample consumption, workflow time, and inter-assay variability. This integrated recovery enables parallel analysis of DNA- and RNA-based biomarkers from a single input, supporting low-input and precious clinical samples. Robust capture of short and ultra-short cfNA, including highly degraded sub-nucleosomal cfDNA fragments, ultra-short single-stranded DNA, miRNAs, isomiRs, tRFs, piRNAs, and EV-associated small RNAs, helps preserve biomarker classes that are often under-recovered by conventional extraction methods. This expanded recovery supports cfDNA fragmentomics, ctDNA signal enrichment, multi-cancer early detection(MCED) and minimal residual disease (MRD) monitoring in low-tumor-burden settings, fetal cfDNA studies, sensitive small RNA analysis, broader liquid biopsy biomarker discovery, and resolves tumor-specific fragmentomic signatures (such as end-motifs and nucleosome footprints) that are routinely lost by conventional extraction methods.

Seamless Downstream Fractionation & NGS Optimization

Following extraction of this kit, the cfNA eluate can be directly processed using the **DeepCatch™ Small Nucleic Acid Fractionation Kit** (See Appendix for details) for size selection. By focusing on specific fragment populations and filtering out uninformative background, this integrated workflow dramatically improves the signal-to-noise ratio and eliminates wasted sequencing reads.

Kit Contents

This kit contains sufficient reagents and materials to perform 32 reactions from ~0.5 mL of plasma or 16 reactions from 1 mL of plasma.

Component	Amount	Storage
DS1 Buffer	6.5 mL	room temperature
Proteinase K	34 mg	2~8°C before reconstitution -20°C after reconstitution
PK reconstitution buffer	1.7 mL	2~8°C
DS2 Buffer	1.4 mL	room temperature
BD3 Buffer	26 mL	room temperature
Beads 4	2 mL	2~8°C, do not freeze
W5 Concentrate*	35 mL	room temperature
SS6 Buffer	25 mL	room temperature
E7 Buffer	1.7 mL	2~8°C
BD8 Buffer	170 µL	2~8°C
Beads 9	70 µL	2~8°C, do not freeze
Elution Buffer	500 µL	2~8°C

* Before first use, add 70 mL of 100% ethanol to W5 Concentrate to prepare W5 Washing Solution.

Precipitates: In cold storage, precipitates may form in DS1, DS2, BD3, W5, or SS6. If this occurs, warm the solutions to 37°C and shake gently until the precipitate dissolves completely.

Materials Required but Not Provided

- **100% ethanol:** ACS or Molecular Biology Grade. Do not use denatured alcohol.
- **Magnetic Stands:** Compatible with 1.5 ~2 mL microcentrifuge tubes and 50 mL tubes.
- **Incubating shaker:** Capable of 62°C incubation and 1000 rpm shaking with 50 mL conical holder.
- **Vortex mixer:** Capable of 1400 rpm shaking with 1.5~2 mL microcentrifuge tubes holder
- **General Lab Equipment:** Centrifuge, and calibrated pipettes.
- **Consumables:** Nuclease-free, low-binding tubes and aerosol-resistant filter tips to prevent sample loss and cross-contamination.

Safety Information

Waste generated during this protocol may contain guanidine salts. Do not mix any kit waste (liquid or solid) with bleach or bleach-containing disinfectants. Mixing guanidine salts with bleach can generate hazardous gases.

Follow the safety instructions, SDS, and waste disposal recommendations provided by the manufacturer of the upstream purification kit. Wear appropriate personal protective equipment and dispose of all waste according to institutional and local regulations.

Protocol

General Guideline:

- The protocol is compatible with both K2-EDTA and Streck Cell-Free DNA Blood Collection Tubes (BCT).
  Note: K_2-EDTA tubes are the recommended tube for collection of whole blood. Centrifuge the blood to cell-free plasma as soon as possible for best results.
- Use RNase/DNase free, low-binding tubes and tips.
- Vortex the magnetic beads thoroughly before each pipetting step to ensure a homogeneous suspension.
- ⚠**CRITICAL:** Accurate pipetting of ethanol is essential. Use Reverse Pipetting when handling ethanol (see Appendix).
- Recommended vortex speed: 1400 rpm.
- Recommended pulse-spin speed: 5000 rpm for 10~30 sec.

Preparation Before First Use:

- Reconstitute Proteinase K: Pulse spin briefly to collect the powder at the bottom of the tube. Add 1.7ml of PK reconstitution buffer to the Proteinase K tube. Vortex to mix thoroughly, store at -20°C after reconstitution.
- Prepare **W5 Washing Solution**: Add 70mL 100% ethanol to 35 mL W5 Concentrate (Final volume: 105 mL). Mix well after adding ethanol. Tightly cap the bottle and store at room temperature.
- Prepare fresh **80% ethanol** using nuclease free water.

Before Each Use of the Kit:

- Set incubating shaker to 62°C for Proteinase K digestion.
- Ensure there is no precipitates in DS1, DS2, BD3, W5, or SS6. If precipitate is present, warm the buffer at 37°C and mix until completely dissolved.
- Pre-warm E7 Buffer and Elution Buffer to 60°C.

Recommendation for Preparation of cell-free plasma:

Collect blood in EDTA tubes, preferably K2-EDTA tubes. Avoid heparin-containing tubes, as heparin may interfere with nucleic acid binding and inhibit downstream enzymatic reactions.

Note: It is recommended to process plasma immediately upon collection via double-spin to minimize gDNA background.

1. **First Spin** (plasma separation): Centrifuge whole blood at 2,000 x g for 10 min at 4°C.
2. Transfer: Carefully transfer the plasma into a new low-binding tube without disturbing the **buffy coat**, the white interface layer containing white blood cells.
3. **Second Spin** (debris removal): Centrifuge the transferred plasma again at 16,000 x g for 10 minutes at 4°C to remove residual cells/debris.
4. Transfer the clarified supernatant to a new tube without disturbing any pellet.
5. Aliquot plasma into freezer-safe tubes and store at **-80°C** until extraction.

Note: If the clarified (cell-free) plasma has been frozen, avoid repeated freeze-thaw cycles. Thaw the plasma gently and keep it on ice only as long as necessary to minimize nucleic acid degradation.

Procedure

Step 1: Digest with Proteinase K

1.1 Set up the digestion reaction in a 50 mL conical tube according to the table below. Add the reagents in the order listed, vortex briefly after each addition.

Cell-free plasma	0.5 mL	1 mL	2 mL	3 mL
DS1	182.5 µL	365 µL	730 µL	1095 µL
	Vortex briefly to mix			
Proteinase K	50 µL	100 µL	200 µL	300 µL
	Vortex briefly to mix			
DS2	37.5 µL	75 µL	150 µL	225 µL
	Vortex briefly to mix			
Expected Total Volume	**770 µL**	**1540 µL**	**3080 µL**	**4620 µL**

Note 1: Always add DS1 before Proteinase K, mixing thoroughly after each addition. Never pre-mix Proteinase K with DS1 or DS2, as this decreases enzyme activity and reduces nucleic acid yield.

Note 2: If the plasma or serum volume is lower than a volume listed, add nuclease-free water to bring the sample to the closest higher volume listed. Immediately add DS1 according to the corresponding column and mix thoroughly. Then add the remaining components in the order specified.

1.2 Incubate at 62°C for 30 min with shaking at 1000 rpm.

1.3 Let stand on benchtop for 15 min.

Step 2: Bind cfNA to Beads

2.1 Add **BD3** and **100% ethanol** according to the table below. Vortex briefly to mix, then incubate on ice for 5 min.

	Starting Cell-free Plasma Volume			
	0.5 mL	**1 mL**	**2 mL**	**3 mL**
BD3	770 µL	1540 µL	3080 µL	4620 µL
100% ethanol	3.2 mL	6.4 mL	12.8 mL	19.2 mL

2.2 Vortex **Beads 4** thoroughly to ensure complete resuspension, then add according to the table below.

	Starting Cell-free Plasma Volume			
	0.5 mL	**1 mL**	**2 mL**	**3 mL**
Beads 4	60 µL	120 µL	240 µL	360 µL

2.3 Shake for 10 min at room temperature at 1000 rpm.

Note: Vigorous shaking is critical for efficient binding.

2.4 Place the tube on a magnetic stand for **at least 10 min** to allow the solution to clear completely. Carefully discard the supernatant without disturbing the bead pellet.

⚠***CRITICAL:*** *A 10 min magnetic separation is required to ensure complete bead capture and maximum nucleic acid yield.*

Step 3: Wash Beads

3.1 Add **W5 Washing Solution** according to the table below.

	Starting Cell-free Plasma Volume			
	0.5 mL	**1 mL**	**2 mL**	**3 mL**
W5 Washing Solution	1.5mL	3 mL	6 mL	9 mL

⚠***CRITICAL:*** *Ensure that 100% ethanol has already been added to the W5 Concentrate prior to use (see* ***Preparation Before First Use****).*

3.2 Shake for 5 min at room temperature at 1000 rpm.

3.3 Place the tube on a magnetic stand for 5 min, or until the solution clears, then carefully discard the supernatant.

3.4 Repeat steps 3.1–3.3 once for a total of two washes.

Step 4: Size Selection

4.1 Carefully remove any residual liquid with a pipette tip, then add 750 µL of **SS6**.

4.2 Select the desired mode (Standard Mode or Discovery Mode). Add **100% ethanol** according to the table below based on the selected mode.

	Standard Mode	Discovery Mode
	Recover ≥15 nt	Recover >10 nt
100% ethanol	1125 µL	1500 µL

4.3 Shake for 5 min at room temperature at 1000 rpm.

4.4 Place the tube on a magnetic stand for 5 min, or until the solution clears. Carefully discard the supernatant without disturbing the bead pellet.

Step 5: Wash in a Microcentrifuge Tube

5.1 Resuspend the bead pellet in 600 µL of **80% ethanol**, then transfer the suspension to a clean 1.5 mL microcentrifuge tube.

5.2 Vortex for 2 min.

5.3 Pulse spin briefly to collect any droplets from the cap and walls of the tube.

5.4 Place the tube on a magnetic stand for 1~2 min, or until the solution clears. Carefully discard the ethanol.

5.5 *(Optional)* Add 600 µL of 80% ethanol directly to the bead pellet in the same tube. Repeat steps 5.2-5.4 once for a total of two washes.

Step 6: Elution

6.1 Carefully remove all residual liquid using a 10 µL pipette tip, then air-dry the bead pellet at room temperature for 5~10 min.

⚠***CRITICAL:*** *Do not over-dry the beads. The beads are sufficiently dry when the pellet surface changes from glossy to matte.*

6.2 Add pre-warmed **E7 Buffer** to the beads according to the table below, vortex for 5 min.

	Starting Cell-free Plasma Volume	
	≤0.5 mL	>0.5 mL
E7 Buffer	50 µL	100 µL

6.3 Pulse spin briefly, then place the tube on a magnetic stand for 2 min, or until the solution clears.

6.4 Carefully transfer the eluate to a clean 1.5 mL microcentrifuge tube. Discard the bead pellet.

Step 7: cfNA Concentrate and Wash

7.1 To the eluate from step 6.4, add the reagents according to the table below.

	Starting Cell-free Plasma Volume	
	≤0.5 mL	>0.5 mL
BD8	5 µL	10 µL
Beads 9	2 µL	4 µL
100% ethanol	60 µL	120 µL

7.2 Vortex for 5 min.

7.3 Pulse spin briefly, then place the tube on a magnetic stand for 1~2 min, or until the solution clears. Carefully discard the supernatant.

7.4 Add 100 µL of **80% ethanol**, and vortex for 2 min.

7.5 Pulse spin briefly, then place the tube on a magnetic stand for 1~2 min, or until the solution clears. Carefully discard the supernatant.

7.6 Repeat step 7.4 -7.5 once for a total of two washes.

Step 8: Elute Concentrated cfNA

8.1 Carefully remove all residual liquid using a 10 µL pipette tip, then air-dry the bead pellet at room temperature for up to 5 min

⚠***CRITICAL:*** *Do not over-dry the beads. The beads are sufficiently dry when the pellet surface changes from glossy to matte.*

8.2 Add 6 µL ~15 µL of pre-warmed **Elution buffer** to the beads, vortex for 5 min.

8.3 Pulse spin briefly, then place the tube on a magnetic stand for 2 min, or until the solution clears.

8.4 Carefully transfer the eluate to a clean 1.5 mL microcentrifuge tube. Discard the bead pellet.

Storage: *Keep purified cfNA on ice for immediate use. For long-term storage, store at -20°C or −80°C.*

Troubleshooting Guide

1. Low cfNA Yield

- **Incompatible anticoagulant:** Use plasma prepared from blood collected in **K2-EDTA tubes** whenever possible. Avoid heparinized plasma. Heparin may interfere with nucleic acid binding and downstream enzymatic reactions.
- **Sample degradation:** Process whole blood to plasma promptly. Avoid multiple freeze-thaw cycles of plasma samples, as this degrades cfNA. *(Note: Natural cfNA levels can vary significantly among individuals.)*
- **Ethanol issues:** Use only 100% ethanol, ACS grade or equivalent. Do not use old, diluted, or denatured ethanol. Use the **reverse pipetting technique** to ensure accurate volume delivery. After adding ethanol to the **W5 Concentrate**, keep the bottle tightly capped to prevent evaporation.
- **Decreased Proteinase K activity:** Do not pre-mix Proteinase K directly with lysis buffers (DS1/DS2). Always add reagents in the specified order and mix thoroughly after each addition. Store reconstituted Proteinase K at -20°C.
- **RNA degradation:** The kit co-purifies cfRNA. Maintain a strict RNase-free environment: wear clean gloves, use RNase-free consumables, keep tubes capped, and minimize sample exposure to room temperature.
- **Improper bead handling:**
    - *Insufficient beads:* Vortex the magnetic beads thoroughly before pipetting to ensure uniform suspension.
    - *Over-drying:* Do not air-dry beads until they crack. Elute immediately when the pellet transitions from a glossy to a matte appearance.
- **Protocol deviations:** Strictly follow all recommended temperatures, shaking speeds (1000 rpm), and reagent volumes. Ensure the Elution Buffer is pre-warmed to maximize recovery.

2. Unreliable Concentration Readings

- **Spectrophotometer limitations:** cfNA yield from plasma or serum is usually low and often below the reliable detection range of NanoDrop or other UV-based methods. A260 values, A260/A280 ratios, and A260/A230 ratios may be unreliable and can be affected by background absorbance.
- **Use appropriate assays:** For routine quantification, use high-sensitivity fluorescence assays (e.g., Qubit). Use a Bioanalyzer or TapeStation to assess fragment size distribution, and qPCR/ddPCR for absolute quantitative measurements.

3. High Genomic DNA (gDNA) Background

- **Cellular carryover:** High gDNA background is usually caused by leukocyte lysis during blood collection or poor plasma separation. Process blood promptly, avoid disturbing the buffy coat during plasma transfer, and do not use hemolyzed or lipemic samples.

4. Poor Downstream Performance

DeepCatch™ is a carrier-free system (no PEG, glycogen, or LPA), eliminating additive interference. If downstream performance is poor, check the following:

- **Residual ethanol:** Ethanol carryover strongly inhibits enzymatic reactions. After the final ethanol wash, remove all residual ethanol with a 10 μL pipette tip. Ensure the bead pellet is air-dried (matte appearance) prior to elution.
- **Salt contamination:** Ensure the 80% ethanol washing steps are performed thoroughly, and all washing buffers are completely removed before drying.

5. Precipitate in Buffers

- **Cold storage:** White precipitates may form in buffers if stored at cold temperatures. Warm the buffers and mix thoroughly until completely clear before use.

Appendix

A. Reverse pipetting technique:

1. Press the plunger to the second stop.
2. Immerse the tip in ethanol and slowly release the plunger to aspirate.
3. Dispense by pressing to the first stop only.
4. Leave the residual ethanol in the tip and discard the tip.

B. Recommended Complete Workflows

For applications requiring size fractionation, purified cfNA from this kit can be further processed using the **DeepCatch™ Small Nucleic Acid Fractionation Kit**. Combining these two kits provides a streamlined end-to-end workflow for targeted enrichment of selected ultra-short nucleic acid fractions.

The **DeepCatch™ Small Nucleic Acid Fractionation Kit** (Cat# GM1016, GM1048) offers three specialized modes for fractionation of ultra-short nucleic acids:

	Mode A *(tRNA Depletion / Small RNA Enrichment)*	**Mode B** *(Ultra-short cfNA Enrichment)*	**Mode C** *(Sub-Nucleosomal cfNA Enrichment)*
Size Cutoff	**Target Enrichment:** Single stranded: 10-40 nt Double stranded: < 25 bp **Background Depletion:** Single stranded: > 40 nt Double stranded: > 25 bp	**Target Enrichment:** 10-60 nt/bp **Background Depletion:** >60 nt/bp	**Target Enrichment:** 10-110 nt/bp **Background Depletion:** >110 nt/bp
Applications	Enriches 10-40 nt small RNAs, including miRNA-sized species, while depleting larger RNA background species (>40 nt), such as full-length tRNAs and longer tRNA-derived RNAs. This reduces background complexity and improves the proportion of informative reads in downstream NGS.	Enriches the ultra-short fraction of cell-free nucleic acids, including cfDNA populations around 50 nt. This supports fragmentomics research by improving access to non-canonical short-fragment populations that may otherwise be underrepresented in conventional workflows.	Enriches sub-nucleosomal and ultra-short nucleic acid fractions by depleting larger fragments, including the dominant mono-nucleosomal cfDNA peak (~167 bp). This improves analytical focus and increases the relative representation of ultra-short fragments in downstream NGS.

C. Safety Information

When working with chemicals, always wear appropriate personal protective equipment (PPE), including a suitable lab coat, disposable gloves, and protective goggles. For detailed safety information, please consult the relevant Safety Data Sheets (SDSs), available online at www.gmbiosciences.com.

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