GM Biosciences

Cat. No. GM1216, GM1248

DeepCatch™ Flow-Through Short Nucleic Acid Recovery Kit (Lysate Compatible)

Recover the missed ultra-short nucleic acid fragments from conventional flow-through or other post-binding fractions

For Research Use Only

Introduction

DeepCatch™ Flow-Through Short Nucleic Acid Recovery Kit (Lysate Compatible) is designed to recover short RNA and DNA fragments from protein-containing flow-through fractions or post-binding supernatants generated by a variety of nucleic acid purification workflows.

This kit is intended for flow-through or post-binding supernatant fractions in which proteins remain present. Typical inputs include: (1) flow-through or supernatant generated from proteinase K digested plasma, serum, cells, tissue, urine, etc., and (2) post-binding supernatants generated from phenol-based lysis workflows without phase separation. These samples may retain proteins, lipids, detergents, chaotropic salts, phenol-containing reagent components, or other sample-derived materials.

Conventional purification methods are effective for many standard nucleic acid applications. However, short nucleic acid fragments may not be fully retained and can be discarded with the flow-through or post-binding supernatant. These missed fragments may include ultra-short cfDNA/cfRNA, miRNA-size RNA, ultra-short RNA, small RNA fragments, and other short nucleic acid species.

This kit uses proprietary DeepCatch™ platform technology to recover missed ultra-short nucleic acids with high efficiency. The workflow is optimized for complex, protein-containing matrices and supports the recovery of short RNA and DNA fragments down to 10 nt. By capturing short nucleic acid fragments that conventional workflows miss, this kit provides an essential recovery layer for low abundance and underrepresented short nucleic acid species. Recovered nucleic acids are suitable for downstream research applications such as RT-qPCR, qPCR, adapter ligation, small RNA profiling, NGS library preparation, and biomarker discovery.

The kit does not require carrier nucleic acids, PEG, glycogen, LPA, or related additives. This helps reduce background and minimizes carryover of materials that may interfere with downstream applications.

Kit Contents

This kit contains sufficient reagents and materials for 16 or 48 reactions. Each reaction is designed to process up to 0.5 mL of flow-through or post-binding supernatant.

Component	GM1216 (16 rxns)	GM1248 (48 rxns)	Storage
DeepCatch™ Beads 1	170 µL	500 µL	2-8°C, do not freeze
W2 Concentrate*	10 mL	30 mL	room temperature
SS3 Buffer	9 mL	25 mL	room temperature
Elution Buffer	450 µL	1300 µL	2-8°C

** See "Preparation Before First Use" for ethanol addition instructions.*

Storage and Stability

- **Shelf Life:** All kit components are stable for 12 months from the date of receipt when stored under the recommended conditions.
- **Handling Precipitates:** During cold shipping or storage, precipitates may form in the W2 Concentrate and SS3 Buffer. If this occurs, incubate the buffer at 37°C and invert gently until all precipitates are fully dissolved before use.

Materials Required but Not Provided

- **100% Ethanol:** ACS or Molecular Biology Grade. Do not use denatured alcohol.
- **Magnetic Stand:** Compatible with 1.5 mL or 2.0 mL microcentrifuge tubes and 10~50 mL tubes.
- **General Lab Equipment:** Benchtop microcentrifuge, vortex mixer, and calibrated pipettes.
- **Consumables:** Nuclease-free, low-bind tubes and filter tips to prevent sample loss and cross-contamination.

Before You Begin

General Guideline

- 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

- **W2 Washing Buffer Preparation:** Add 100% ethanol to the **W2 Concentrate** to prepare the working **W2 Washing Buffer**. Mix well after adding ethanol. Tightly cap the bottle and store at room temperature.

  For GM1216 (16 rxns): add 20 mL of 100% ethanol to 10 mL W2 Concentrate (Final volume: 30 mL).
  For GM1248 (48 rxns): add 60 mL of 100% ethanol to 30 mL W2 Concentrate (Final volume: 90 mL).

- **Prepare 80% ethanol using nuclease-free water**

Before Each Use of the Kit

- Ensure there is no precipitates in **W2 Washing Buffer** and **SS3 Buffer**. If precipitate is present, warm the buffer and mix until completely dissolved.
- Pre-warm Elution Buffer to 60°C.

Compatible Starting Materials

This kit is designed for protein-containing flow-through fractions or post-binding supernatants generated from the following workflows.

1. **Proteinase K–Digested Samples**

   Compatible inputs include flow-through or post-binding supernatant generated during nucleic acid purification from Proteinase K–digested plasma, serum, cells, tissue, urine, etc.

The upstream workflow may use a column, filter, or magnetic beads to bind the primary nucleic acid fraction. Short RNA and DNA fragments that are not retained during the binding step may remain in the flow-through or post-binding supernatant and can be recovered using this kit.

These fractions may contain residual proteins, lipids, detergents, chaotropic salts, alcohol, or other components derived from sample processing.

2. **Phenol-Based Lysis Without Phase Separation**

   Compatible inputs also include post-binding supernatants generated from samples lysed with a phenol-based reagent, such as TRIzol or TRI Reagent, without chloroform phase separation.

   In this workflow, alcohol is added directly to the lysate, and magnetic beads are used to bind longer nucleic acids. The resulting post-binding supernatant, which may retain short and ultra-short nucleic acid fragments, is used as the starting material for this kit.

   Because chloroform phase separation is not performed, the supernatant may contain residual proteins, phenol, guanidinium salts, lipids, alcohol, and other lysis-derived components.

3. **Potentially Compatible Workflows (Exploratory Use)**

   This kit may also be used to recover short nucleic acids from the following sample types:

   - Direct Guanidine Lysates (No PK Digestion): Flow-through or post-binding supernatants generated from samples directly lysed in guanidinium thiocyanate (GITC) without prior Proteinase K digestion, followed by the addition of alcohol and initial long-nucleic acid captured via silica columns or magnetic beads.
   - Chemically Precipitated Guanidine Lysates: Flow-through or post-binding supernatants derived from GITC-based lysates where proteins were depleted via chemical precipitation and centrifugation, prior to the addition of alcohol and primary column or bead capture.

   Important Considerations for Exploratory Samples: While the flow-through or post-binding supernatants from these workflows are viable candidates for short nucleic acid recovery, performance may vary significantly depending on the original starting material (e.g., exosome pellets, eukaryotic cells, tissues, bacteria, fungi, plants, environmental samples, and other complex sources).

   Empirical optimization may be required to address matrix-specific contaminants such as residual proteins, cell debris, lipids, polysaccharides, pigments, or downstream enzymatic inhibitors. Processing these highly variable matrices is at the user's discretion.

This kit can only recover short nucleic acid fragments that are present in the input unbound fractions. Targets already lost in earlier upstream steps cannot be Recovered.

Safety Information

Flow-through or post-binding supernatant may contain residual phenol, chloroform, guanidinium salts, or other lysis reagents from upstream purification workflows. Do not add bleach or bleach-containing disinfectants to these samples or waste containing these materials.

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

Step 1: Collect and Measure the Flow-Through or Post-Binding Supernatant

1.1 Follow the protocol of the upstream third-party purification kit. Collect the resulting flow-through (from column/filter workflows) or post-binding supernatant (from magnetic bead workflows).

1.2 Transfer the flow-through/post-binding supernatant to a new tube. Measure or estimate the total volume of the flow-through/post-binding supernatant and record this volume as A µL.

1.3 Calculate the volume of ethanol already present in the flow-through/post-binding supernatant. Record this as B µL. This value will be used to calculate the amount of additional ethanol required in Step 2.

Note: If your upstream purification workflow utilized isopropanol instead of ethanol, treat the volume of isopropanol identically to ethanol. Simply use the isopropanol volume as Volume B in all calculations below.

Step 2: Recover Short Nucleic Acids

2.1 Calculate and add (2A-3B) µL 100% ethanol to the flow-through/post-binding supernatant.

Example Calculation:

For a 500 µL flow-through volume (A) containing 275 µL ethanol (B): (2 × 500) – (3 × 275) = 175 µL. Add 175 µL 100% ethanol to the 500 µL flow-through.

Note:

- ***Unknown Alcohol Volume:*** *If the flow-through or post-binding supernatant contains alcohol but the exact alcohol volume (B) cannot be determined, add 0.2 × A µL of 100% ethanol as an initial adjustment. If recovery is suboptimal, empirically optimize the added ethanol volume within a range of 0.2 × A to 0.8 × A µL. Optimal conditions may vary depending on the upstream purification chemistry and sample matrix, particularly for critical samples or non-standard workflows. Under these conditions, Standard Mode and Discovery Mode cannot be reliably selected.*
- ***Handling****: Use the reverse pipetting technique when handling ethanol to ensure volumetric accuracy (see Appendix A).*

2.2 Add 10 µL of fully resuspended **DeepCatch™ Beads 1** for starting flow-through or post-binding supernatant volumes (recorded as A µL in Step 1.2) of up to 500 µL.

Note: For starting volumes greater than 500 µL, increase the bead volume proportionally at a ratio of 2 µL per 100 µL of starting material (e.g., add 14 µL of beads for a 700 µL sample).

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 **5 min** to allow the solution to clear completely. Carefully discard the supernatant without disturbing the bead pellet.

⚠***CRITICAL:*** *A 5 min magnetic separation is required to ensure complete bead capture and maximum nucleic acid yield.*

Step 3: Wash the Beads

3.1 Add 900 µL **W2 Washing Buffer**.

⚠ ***CRITICAL:*** *Ensure that 100% ethanol has already been added to the W2 Concentrate prior to use (see* ***Preparation Before First Use****).*

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

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

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

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

Step 4: Size Selection

4.1 Carefully remove any residual liquid with a pipette tip, then add 500 µL of **SS3**.

4.2 The kit provides two user-selectable options for recovery down to either 15 nt or 10 nt nucleic acid. Add **100% ethanol** according to the table below based on the selected option.

	100% ethanol
Standard Mode (Recover ≥15 nt)	750 µL
Discovery Mode (Recover >10 nt)	1,000 µL

Note: If the exact alcohol volume of the starting material was unknown and empirically adjusted (as described in Step 2.1), precise size-fractionation cannot be reliably achieved. In such cases, users may proceed by selecting either mode, though the specific size cutoff (15 nt or 10 nt) is not guaranteed.

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: Ethanol Washes

5.1 Resuspend the bead pellet in 750 µL of 80% ethanol, then transfer the suspension to a clean 1.5 mL microcentrifuge tube.

Note: For post-binding supernatants generated from "Phenol-Based Lysis Without Phase Separation", use 90% ethanol instead of 80% ethanol.

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 Add 750 µL of 80% ethanol directly to the bead pellet in the same tube. Repeat Steps 5.2–5.4 for a total of two ethanol washes.

Note: For post-binding supernatants generated from "Phenol-Based Lysis Without Phase Separation", perform a total of three washes using 90% ethanol.

Step 6: Elute the Recovered Nucleic Acids

6.1 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.*

6.2 Add 10 µL ~ 25 µL of pre-warmed Elution Buffer to the beads, vortex for 5 min.

Note: For starting flow-through or post-binding supernatant volumes (recorded as A µL in Step 1.2) greater than 500 µL, increase the selected Elution Buffer volume proportionally based on the starting volume.

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 RNase/DNase free microcentrifuge tube. Discard the bead.

Note (Optional Concentration): For samples with a large initial input volume (recorded as A µL in Step 1.2) resulting in a proportionally large elution volume (e.g., a 2 mL starting sample requiring 40-100 µL of Elution Buffer), the final nucleic acid concentration may be dilute. If further concentration is required, the ***DeepCatch™ Broad-Range Nucleic Acid Concentration Kit (Cat. No.: GM1348)*** *can be used to concentrate ~200 µL of eluate to a final volume as low as 5 µL.*

Elution and Storage Guidelines

Sample Pooling (For Total Nucleic Acid Profiling): The eluate generated by this kit contains only the ultra-short nucleic acid fraction missed by conventional methods. To obtain a complete, total nucleic acid profile, you must combine this eluate with the eluate from your primary purification column (which contains the larger fragments).

Downstream tRNA Depletion: If your pooled sample contains abundant tRNA and your downstream application requires the near-complete depletion of tRNA and other background RNAs, we highly recommend further processing the sample using **DeepCatch™ Small Nucleic Acid Fractionation Kit** (Cat. No. GM1016, GM1048).

Nucleic Acid Quantification: See Appendix B for guideline.

Storage Conditions: Keep the eluted nucleic acids on ice for immediate use in downstream applications. For long-term storage, store the eluate at -80°C.

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. Guidelines for Nucleic Acid Quantification

Recovered short nucleic acids may be present at very low concentrations and are often below the reliable detection range of standard quantification methods (NanoDrop™ or other UV-based spectrophotometric methods). In these cases, concentration readings, A260 values, and A260/A280 or A260/A230 ratios may be inaccurate and may be affected by background absorbance from salts, buffers, or other sample components.

For accurate measurement of low-yield short nucleic acids, we strongly recommend using the following target-specific assays:

- **Routine Concentration Measurement:** Use high-sensitivity fluorometric assays (e.g., Qubit™ microRNA or ssDNA Assays). Fluorescence-based assays are generally more reliable than UV absorbance for low-input nucleic acids. However, standard fluorescence assays are typically optimized for longer DNA/RNA and may show reduced or nonlinear signal response to ultra-short fragments, especially in the 10–40 nt range. Therefore, a low or undetectable Qubit™ reading does not necessarily indicate poor recovery of ultra-short fragments.
- **Fragment Size & Distribution:** Use capillary electrophoresis platforms (e.g., Agilent Bioanalyzer® or TapeStation®) equipped with high-sensitivity small RNA or ssDNA chips to accurately profile the short fragment distribution.
- **Absolute Quantification:** Use targeted qPCR or digital droplet PCR (ddPCR) for the most precise, absolute measurement of specific short fragments (e.g., target miRNAs or ultra-short cfDNA).

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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