GM Biosciences

Cat. No. GM1116, GM1148

DeepCatch™ Flow-Through Short Nucleic Acid Recovery Kit (Organic Extracted)

Recover the missed ultra-short nucleic acid fragments from conventional flow-through

For Research Use Only

Introduction

DeepCatch™ Flow-Through Short Nucleic Acid Recovery Kit (Organic Extracted) is designed to recover ultra-short RNA and DNA fragments from the flow-through of conventional column/filter-based purification systems. It is intended for flow-through generated from workflows that include phenol/chloroform, acid-phenol/chloroform, TRIzol, QIAzol, or similar organic extraction steps. In these workflows, proteins and many other sample-associated contaminants are largely removed during phase separation, generating a protein depleted flow-through.

While standard purification kits are highly optimized for capturing total RNA, small RNA, cfRNA, or cfDNA, short nucleic acids are frequently under-recovered and discarded with the flow-through. Conventional kits often show size-dependent loss across the 10–35 nt range, where many biologically important small RNA species are found, including miRNAs, siRNAs, tRFs, and piRNA-size fragments. Loss becomes especially pronounced below 20 nt.

DeepCatch™ captures the missed short-fragment fraction from flow-through samples by using proprietary flexible-interface magnetic bead technology. This enables more complete recovery of short RNA and DNA fragments that may be under-captured by conventional purification workflows.

The kit is suitable for recovery of under-captured short RNA and DNA fragments, including ultra-short RNA, miRNA-size RNA, tRFs, piRNA-size fragments, cfRNA, and ultra-short cfDNA. All recovered nucleic acids are ready for downstream research applications, including 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 500 µL of flow-through.

Component	GM1116 (16 rxns)	GM1148 (48 rxns)	Storage
DeepCatch™ Beads	300 µL	900 µL	2-8°C, do not freeze
Washing Concentrate*	10 mL	30 mL	room temperature
Elution Buffer	350 µL	1050 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 Washing Concentrate. 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.
- **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

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

  For GM1116 (16 rxns): add 20 mL of 100% ethanol to 10 mL Washing Concentrate (Final volume: 30 mL).
  For GM1148 (48 rxns): add 60 mL of 100% ethanol to 30 mL Washing Concentrate (Final volume: 90 mL).

- Prepare 80% ethanol using nuclease-free water

Before Each Use of the Kit

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

Compatible Starting Materials

Flow-through generated directly after loading the aqueous phase of an organic-extracted sample (phenol/chloroform, acid-phenol/chloroform, TRIzol, QIAzol, or a similar organic extraction method) onto a column.

Incompatible Sample Types
Do not use this kit for flow-through fractions or post-binding supernatants generated from workflows in which proteins were not removed by phenol/chloroform phase separation. This includes:
• **Phenol-based lysis without chloroform phase separation:** Samples lysed with TRIzol, TRI Reagent, QIAzol, or a similar phenol-based reagent, followed by direct addition of isopropanol and magnetic-bead binding. The resulting post-binding supernatant should not be processed with this kit.
• **Guanidinium thiocyanate lysis followed by protein precipitation:** Samples lysed with a GITC-based reagent and treated with a protein precipitation reagent, followed by centrifugation to remove the precipitated material. Then, the recovered supernatant is subsequently mixed with isopropanol and

processed using a column or magnetic beads. The resulting flow-through or post-binding supernatant should not be processed with this kit.

For the above two sample types, use the Lysate Compatible version (Cat. No.: GM1216, GM1248).

This kit can only recover short nucleic acid fragments that are present in the input flow-through. Targets already lost in earlier upstream steps cannot be recovered.

Safety Information

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

1.1 Following the protocol of upstream column/filter-based purification systems to generate flow-through.

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

***Note**: A standard reaction is designed for up to approximately 500 µL of flow-through.*

1.3 Calculate the volume of ethanol already present in the flow-through. 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 The kit provides two user-selectable options for recovery down to either 15 nt or 10 nt. Select the desired recovery mode and calculate the volume of 100% ethanol to add to the flow-through using the formula below. Add the calculated volume of ethanol to the flow-through.

Recovery Mode	Recovery Range	Ethanol to Add
Standard Mode	≥15 nt	1.5A – 2.5B
Discovery Mode	>10 nt	2A – 3B

Example Calculation:

For a 400 µL flow-through volume (A) containing 220 µL ethanol (B):

- *Standard Mode: (1.5 × 400) – (2.5 × 220) = 50 µL additional ethanol.*
- *Discovery Mode: (2.0 × 400) – (3.0 × 220) = 140 µL additional ethanol.*

Note:

- ***Unknown Alcohol Volume:** If the flow-through contains ethanol or isopropanol 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 18 µL of fully resuspended **DeepCatch™ Beads** for starting flow-through 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 (e.g., add 27 µL of beads for a 750 µL sample).*

2.3 Vortex for 5 min.

2.4 Place the tube on a magnetic stand for 5 min, or until the solution is clear. Carefully remove and discard the supernatant without disturbing the bead pellet.

Step 3: Wash the Beads

Washing Buffer Wash

3.1 Add 900 µL of **Washing Buffer**.

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

3.2 Vortex for 2 min.

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 1~2 min, or until the solution clears. Carefully discard the supernatant.

3.5 *(Optional)* Repeat Steps 3.1-3.4 once for a total of two washes.

80% Ethanol Wash

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

3.7 Vortex for 2 min.

3.8 Pulse spin briefly to collect any droplets.

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

3.10 Add 600 µL of 80% ethanol directly to the bead pellet in the same tube. Repeat steps 3.7-3.9 once for a total of two washes.

Step 4: Elute the Nucleic Acids

4.1 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.*

4.2 Add 6 µL ~ 20 µL of pre-warmed **Elution Buffer** to the beads, vortex for 5 min.

Note: *For starting 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.*

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

4.4 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 24-80 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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