Cat. No. GM1016, GM1048

DeepCatch™ Small Nucleic Acid Fractionation Kit

tRNA Depletion · Small RNA Enrichment · Ultra-Short cfNA Fractionation

For Research Use Only

Introduction

DeepCatch™ Small Nucleic Acid Fractionation Kit is designed for use with purified nucleic acids (NAs). The kit enables highly efficient enrichment of ultra-short fragments while selectively depleting unwanted background noises. By reducing non-informative background NAs, the kit improves the efficiency of downstream workflows such as qPCR, library preparation, and next-generation sequencing (NGS). This allows final readouts to be more focused on biologically meaningful targets.

Key applications include:

- **Targeted Enrichment:** Highly efficient capture of ultra-short nucleic acids.
- **Small RNA Optimization:** Depletion of tRNA from small RNA preparations (e.g., miRNA, Y RNA, and tRFs).
- **cfDNA Fragmentomics:** Revealing previously hidden cfNA fragment populations by selectively enriching ultra-short cfNA and depleting the dominant mono-nucleosomal background.

The DeepCatch™ platform is based on a proprietary flexible-interface magnetic bead technology that enables highly efficient enrichment and high-resolution fractionation of ultra-short nucleic acids. This technology offers several key advantages for nucleic acid preparation:

- **Unmatched Short-Fragment Recovery:** Efficiently enriches nucleic acids as short as 10 nt, unlocking targets that are otherwise inaccessible.
- **Optimal Downstream NGS Compatibility:** Completely eliminates the need for carrier NAs, PEG, glycogen and LPA, and related additives that may interfere with downstream enzymatic reactions, helping provide cleaner input for library preparation and sequencing.

The kit provides three specialized modes for precision 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/bp. 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.

* The effective cutoff depends not only on fragment length, but also on strand state, molecular structure, and sample composition. Therefore, the cutoff values listed for each mode should be considered nominal guide values rather than absolute boundaries. In general, each mode includes a transition zone in which partial recovery of larger fragments may occur.

Mode A: The transition zone is approximately 40-50 nt. The nominal enriched fraction for single-stranded nucleic acids is 10-40 nt, although partial recovery of fragments around 50 nt may be observed depending on sample type and composition. Under the same conditions, double-stranded nucleic acids show different fractionation behavior, and dsDNA fragments larger than ~25 bp are depleted.

Mode B: The transition zone is approximately 60-100 nt/bp. The nominal enriched fraction is 10-60 nt/bp, although partial recovery of fragments up to 100 nt/bp may be observed depending on sample type and composition.

Mode C: The nominal upper cutoff is ~110 nt/bp. The effective upper cutoff may vary slightly among samples and is typically observed within the range of 100-120 nt/bp.

Kit Contents

This kit provides sufficient reagents for 16 or 48 reactions, based on a standard input of 25 µL containing up to 2 µg of nucleic acid per reaction.

Component	GM1016 (16 rxns)	GM1048 (48 rxns)	Storage
D40 Buffer	1.4 mL	1.4 mL x 3	room temperature
D60 Buffer	1.4 mL	1.4 mL x 3	room temperature
D110 Buffer	1.4 mL	1.4 mL x 3	room temperature
Beads 1	60 µL	60 µL x 3	2-8°C, do not freeze
Beads 2	80 µL	80 µL x 3	2-8°C, do not freeze
Elution Buffer	900 µL	900 µL x 3	2-8°C

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 D40, D60 and D110 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.
- **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.

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

Before You Start:

- 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 for precise fractionation. Use **Reverse Pipetting** when handling ethanol (see Appendix).
- Ensure there is no precipitates in **D40, D60 and D110 Buffer**. If precipitate is present, warm the buffer and mix until completely dissolved.
- Recommended vortex speed: **1400 rpm**.

- Recommended pulse-spin speed: **5000 rpm for 10-30 seconds**.
- Use freshly prepared **80% ethanol** with nuclease-free water.
- Pre-warm Elution Buffer to **60°C**.

Compatible Starting Materials:

- **Purified nucleic acids**: in RNase/DNase-free 1x Low TE, 1x TE, or water.
- **Direct Enzymatic Reactions**: Unpurified reactions (such as ligation, DNase/RNase treatment, or amplification) can be directly loaded.

> **Important Input Considerations for Cellular/Tissue Total RNA:** Target ultra-short RNAs (e.g., miRNAs) represent a minute fraction, typically only **0.01%–0.1%** of total cellular RNA. Since a single standard reaction takes up to 2 µg total RNA, a single prep may not provide enough yield for downstream use. Please estimate your required final mass and scale up the reaction proportionally.

> **You cannot enrich what is already lost.** Conventional kits routinely wash away fragments under 20 nt. To ensure these ultra-short targets are retained for fractionation, we highly recommend preparing your initial samples with DeepCatch™ Isolation Kits, which is engineered to reliably capture nucleic acids down to 10 nt. See Appendix for details.
>
> Alternatively, if a conventional kit is used, keep the flow-through and recover the missed ultra-short nucleic acids using the DeepCatch™ Flow-Through Recovery Kits (Cat# GM1116, GM1148, GM1216, GM1248).

Step 1: Long Fragment Depletion/Fractionation

Steps	Mode A	Mode B	Mode C
1.1	Add 25µL (≤2ug) starting material to a microcentrifuge tube.	Add 25µL (≤2ug) starting material to a microcentrifuge tube.	Add 25µL (≤2ug) starting material to a microcentrifuge tube.
1.2	Add 75 µL **D40** Buffer and 3.5 µL well-vortexed **Beads 1**.	Add 75 µL **D60** Buffer and 3.5 µL well-vortexed **Beads 1**	Add 75 µL **D110** Buffer and 3.5 µL well-vortexed **Beads 1**
1.3	Add 100% ethanol according to sample type: • **130 µL** ethanol for tRNA-rich samples (tissue/cell derived) • **150 µL** ethanol for tRNA-poor samples (plasma/serum derived)	Add **60 µL** 100% ethanol.	Add **30 µL** 100% ethanol.

Steps	All Modes
1.4	Vortex for 5 min.
1.5	Put the tube on a magnetic stand for 1-2 min, or until the solution is clear. Transfer the supernatant to a new tube and discard the beads. ⚠**CRITICAL:** The supernatant contains your target ultra-short fragments. **KEEP SUPERNATANT.** 📝 (OPTIONAL) Recovery of Depleted Long Fragments. If you wish to recover the long nucleic acids bound to the Beads 1, do not discard them. Instead, retain the beads and proceed directly to Step 3 (Washing) and

Step 4 (Elution). *Pro Tip: To ensure optimal recovery and prevent the beads from drying out, immediately add 200 µL of 80% ethanol to the bead pellet once the supernatant is removed.*

Step 2: Short Fragment Enrichment

Steps	Mode A	Mode B	Mode C
2.1	Add 100% ethanol according to the sample type from Step 1.3: • **70 µL** for tRNA-rich samples (tissue/cell derived) • **50 µL** for tRNA-poor samples (plasma/serum derived)	Add **140 µL** 100% ethanol.	Add **170 µL** 100% ethanol.

Steps	All Modes
2.2	Add 4.5 µL well-vortexed **Beads 2**
2.3	Vortex for 5 min.
2.4	Pulse spin, then put the tube on a magnetic stand for 1-2 min, or until the solution is clear. Discard the supernatant without disturbing the beads.

Step 3: Washing

Steps	All Modes
3.1	Resuspend the bead pellet in 200 µL of 80% ethanol, then transfer the suspension to a clean 1.5 mL microcentrifuge tube.
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 is clear, and discard the supernatant without disturbing the beads.
3.5	Add 200 µL of 80% ethanol directly to the bead pellet in the same tube, repeat step 3.2-3.4 one more time for a total of two washes.

Step 4: Elution

Steps	All Modes
4.1	Remove any residual ethanol using a 10 µL pipette tip, and air-dry the beads at room temperature for up to 5 minutes. ⚠**CRITICAL:** The beads are sufficiently dry when the pellet surface transitions from a glossy to a matte appearance. Avoid over-drying, as this may lead to poor resuspension and significantly reduced elution efficiency.
4.2	Add 6 µL - 25 µL of pre-warmed **Elution Buffer** (60°C) and vortex for 5 min.
4.3	Pulse spin, place the tube on a magnetic stand to pellet the beads for 1-2 min, or until the solution is clear, and transfer the supernatant to a new tube.

Elution and Storage Guidelines

Volume Optimization: The standard elution volume is 6–25 µL. If the expected nucleic acid yield is low and a higher downstream concentration is required, you may reduce the elution volume to a minimum of 6 µL.

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. Recommended Complete Workflows

Conventional extraction methods routinely lose ultra-short fragments. DeepCatch™ Isolation Kits listed below are designed to efficiently recover ultra-short nucleic acids down to 10 nt directly from biological samples. When used together with the DeepCatch™ Isolation Kits, this fractionation kit provides a streamlined end-to-end workflow for optimal recovery and profiling of both small RNAs and ultra-short cfNAs.

Workflow Type	Upstream Kit	Downstream Kit	Unique Features
Small RNA Workflow	DeepCatch™ Small RNA Isolation Kit (GM3036)	This Kit	• High-yield isolation of ultra-short RNA • User-selectable lower limits (10 nt or 15 nt) • Unmatched tRNA depletion efficiency
Ultra-short cfNA Workflow	DeepCatch™ Cell-free Total Nucleic Acids Isolation Kit (Plasma/Serum) (GM2016)	This Kit	• High-yield isolation of ultra-short cfNA • User-selectable lower limits (10 nt or 15 nt) • cfDNA fragmentomics

C. Guidelines for Nucleic Acid Quantification

Enriched 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).

D. 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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