Cat. No. GM1348

DeepCatch™ Broad-Range Nucleic Acid Concentration Kit

Carrier-free concentration of purified DNA and RNA for low-input NGS and sensitive downstream assays without sacrificing ultra-short cfNAs and small RNAs

For Research Use Only

Introduction

DeepCatch™ Broad-Range Nucleic Acid Concentration Kit is designed to rapidly concentrate purified DNA and RNA into ultra-low volumes while providing high recovery across a broad size range, including ultra-short fragments down to 10 nt. Developed for downstream assays where target nucleic acids are extremely dilute or of exceedingly low abundance, this carrier-free system maximizes the final sample concentration without introducing exogenous nucleic acid interference.

Low-input nucleic acid samples may require concentration before use in downstream molecular assays. In research workflows involving liquid biopsy, noninvasive prenatal screening, extracellular-vesicle profiling, and other biofluid-derived samples, cfDNA, cfRNA, miRNAs, and other target nucleic acids may be recovered at picogram-to-nanogram levels in relatively large sample volumes. However, downstream assays such as NGS, ddPCR, qPCR, and RT-qPCR accept only limited sample volumes. Therefore, only a portion of the available nucleic acid may be introduced into each reaction unless the sample is concentrated. Conventional concentration methods may not fully address this limitation. Alcohol precipitation is time-consuming and may result in substantial loss of ultra-short fragments. Vacuum evaporation methods, such as SpeedVac concentration, may expose nucleic acids to heat and concentrates salts and other potential assay inhibitors together with the targeted nucleic acids. Standard clean-up kits may also show reduced recovery of fragments below 20 nt.

This kit addresses these limitations using proprietary DeepCatch™ magnetic bead technology to capture both single- and double-stranded DNA and RNA across a broad size range. Samples with starting volumes of up to 200 µL can be eluted in as little as 5 µL, providing up to a 40-fold reduction in sample volume, while wash steps help reduce residual soluble impurities. In representative applications, nucleic acid samples at pg/µL-level starting concentrations can be concentrated to ng/µL-level concentrations and, under suitable conditions, to concentrations in the tens of ng/µL. The resulting eluate is highly concentrated and purified, with broad-range retention of short and ultra-short nucleic acids under the tested conditions, allowing critical short nucleic acids to be retained for immediate downstream use.

Typical Applications

By reducing the final sample volume, the kit enables a greater proportion of the available nucleic acid to be added to downstream reactions with strict input-volume limits. Recommended applications include:

- Low-input NGS workflows and selected PCR-free NGS applications
- Liquid biopsy library preparation using cfDNA and cfRNA
- Small RNA profiling, including plasma miRNA, EV-derived RNA, and low-abundance RNA from cerebrospinal fluid or urine
- ddPCR assays for rare-mutation and low-abundance target detection
- High-sensitivity qPCR and RT-qPCR analysis of low-abundance targets

While fully compatible with nucleic acids purified using third-party systems, to maximize the retention of ultra-short targets, the kit can be used downstream of other DeepCatch™ products, including the DeepCatch™ Small Nucleic Acid Fractionation Kit and DeepCatch™ nucleic acid isolation kits.

Kit Contents

This kit contains sufficient reagents and materials for 48 reactions. Each reaction can process up to 200 µL of purified nucleic acid solution containing no more than 1 µg of total nucleic acid.

Component	GM1348 (48 rxns)	Storage
DeepCatch™ Binding Buffer	1,000 µL	2-8°C, do not freeze
DeepCatch™ Beads	500 µL	2-8°C, do not freeze
Elution Buffer	800 µL	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 DeepCatch™ Binding 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.

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

- Prepare 80% ethanol using nuclease-free water

Before Each Use of the Kit

- Pre-warm Elution Buffer to 60°C.

Compatible Starting Materials

The DeepCatch™ Broad-Range Nucleic Acid Concentration Kit is intended for previously purified DNA and/or RNA dissolved in one of the following:

- Low-TE buffer, pH≤8.5
- TE buffer, pH≤8.5

- Tris-HCl, pH≤8.5
- Nuclease-free water

The starting material may contain RNA and/or DNA, including single-stranded and/or double-stranded nucleic acids across a broad size range.

This kit is designed to concentrate both long and short nucleic acids, including fragments as short as 10 nt. For optimal recovery of the complete nucleic acid size range, the upstream purification method must efficiently recover ultra-short nucleic acids. Conventional purification methods that lose short fragments cannot be compensated for during the subsequent concentration step. For best results, use an upstream method validated for ultra-short nucleic acid recovery, such as a DeepCatch™ nucleic acid isolation kit (GM2016, GM3016), flow-through recovery kit (GM1116/GM1148, GM1216/GM1248) or fractionation kit (GM1016/GM1048).

Protocol

Step 1: Binding

1.1 Transfer 200 µL of purified nucleic acid sample to a clean 1.5 mL nuclease-free microcentrifuge tube.
__Note:__ For starting volumes smaller than 200 µL, add nuclease-free water or lowTE to bring the volume to 200 µL.

1.2 Add the following components to the sample according to the table below. *Ensure that the DeepCatch™ Beads are fully resuspended by vortexing before use.*

Component	Volume
Nucleic Acid Sample (from Step 1.1)	200 µL
DeepCatch™ Binding Buffer	20 µL
DeepCatch™ Beads	10 µL
100% Ethanol	345 µL
Total volume	***575 µL***

1.3 Vortex for 5 min to allow the nucleic acids to bind to the beads.

1.4 Pulse spin briefly to collect any droplets.

1.5 Place the tube on a magnetic stand for 2~3 min, or until the solution is clear. Carefully remove and discard the supernatant without disturbing the bead pellet.

Step 2: Washing

2.1 Add 500 µL of **80% ethanol**.

2.2 Vortex for 1 min.

2.3 Pulse spin briefly to collect any droplets.

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

2.5 Repeat Steps 2.1~2.4 once for a total of two ethanol washes.

Step 3: Elution

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

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

3.2 Add 5~15 μL of pre-warmed **Elution Buffer** to the beads, vortex for 2~5 min.

3.3 Pulse spin briefly, then place the tube on a magnetic stand for 1 min, or until the solution clears.

3.4 Carefully transfer the eluate to a clean 1.5 mL RNase/DNase free microcentrifuge tube. Discard the bead.

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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