Cat. No. GM3036

DeepCatch™ Small RNA Isolation Kit

- Enrich <100 nt RNA while efficiently recovering the ultra-short 10-40 nt or 15-40 nt fraction
- Optional recovery of total RNA

For Research Use Only

Introduction

The **DeepCatch™ Small RNA Isolation Kit** is designed for isolation and enrichment of small RNA from a variety of sample input, including tissues, cells, exosomes from plasma/serum/urine/cerebrospinal fluid, yeasts and bacteria. The workflow combines conventional phenol/chloroform phase separation, such as TRIzol/TRI Reagent based RNA extraction, with DeepCatch™ magnetic beads. After phase separation, small RNA in the aqueous phase is selectively enriched. The purified RNA is suitable for common downstream research applications, including RT-qPCR, small RNA profiling, adapter ligation, and NGS library preparation, and can be used for analysis of microRNAs, piRNAs, tRNA-derived fragments, snoRNA-derived fragments, Y RNA fragments, and other short RNA fragments.

Conventional RNA isolation methods often show a sharp loss of RNA fragments below 20 nt. This limitation can lead to under-representation of ultra-short RNA fragments in downstream analysis. The **DeepCatch™ Small RNA Isolation Kit** is developed to overcome this limitation. The kit enables efficient recovery of small and ultra-short RNA fragments down to 10 nt, with typical recovery of ~90% under optimized conditions.

The kit does not require carrier RNA, glycogen, linear polyacrylamide, or other nucleic acid carriers for RNA capture. This **carrier free** design helps reduce artificial background and improves compatibility with downstream applications, including adapter ligation and NGS library preparation.

The Flex-Range protocol allows users to select the RNA recovery range that best fits their study.

Mode	RNA Size Range	Recommended Use
Standard Mode	≥15 nt	For users who focus on conventional small RNA profiling and prefer to exclude fragments below 15 nt.
Discovery Mode	>10 nt	For users who require broader recovery of novel ultra-short biomarkers, including 10–15 nt fragments.

In addition, the kit includes an enrichment step to reduce a large portion of longer RNA fragments (>100 nt). Unlike conventional <200 nt small RNA enrichment workflows, this step increases the relative abundance of short and ultra-short RNA in the final eluate and helps improve downstream small RNA analysis. For applications that require near-complete depletion of longer RNA species, such as tRNA, this kit can be paired with the **DeepCatch™ Small Nucleic Acid Fractionation Kit** (see Appendix for detail). This combined dual-kit approach provides a more complete solution for precise ultra-short RNA enrichment (10-40 nt), including up to 90% removal of tRNA and other background noise. This improves signal-to-noise and supports accurate expression profiling. Especially for Next Generation Sequencing (NGS), reduced tRNA carryover helps minimize waste reads and increases the fraction of informative data, improving overall cost efficiency.

Kit Contents

This kit contains sufficient reagents and materials to perform 36 reactions, each starting from 1 mL of Lysis Reagent such as TRIzol, TRI Reagent, or an equivalent acid phenol–chloroform reagent.

Component	Amount (36 rxns)	Storage
Resuspension Buffer	2 x 1 mL	room temperature
R1 Concentrate*	7.5 mL	room temperature
R2 Beads	0.75 mL	2-8°C, do not freeze
R3 Beads	1.4 mL	2-8°C, do not freeze
W4 Concentrate#	23 mL	room temperature
Elution Buffer	2 x 1 mL	2-8°C

* Before first use, add 22.5 mL of 100% ethanol to R1 Concentrate to prepare **R1 Binding Buffer**.

Before first use, add 46 mL of 100% ethanol to W4 Concentrate to prepare **W4 Washing Buffer**.

Shelf Life: When stored under proper conditions, the kit is stable for 12 months from the date of receipt.

Precipitates: In cold storage, precipitates may form in the Resuspension Buffer, R1 Binding Buffer or W4 Washing Buffer. If this occurs, warm at 37°C and shake gently until the precipitate dissolves completely.

Materials Required but Not Provided

- **Lysis Reagent**: TRIzol Reagent, TRI Reagent, or an equivalent acid phenol–chloroform reagent.
- **100% Ethanol**: ACS or Molecular Biology Grade. Do not use denatured alcohol.
- **Magnetic Stands**: Compatible with 1.5 ~2 mL microcentrifuge tubes.
- **Thermal block or Incubator**: Capable of 60°C incubation with 1.5~2 mL microcentrifuge tubes 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.

Important Prerequisites

- This kit is optimized for use with phenol-based Lysis Reagents (e.g., TRI Reagent, TRIzol), which contain phenol. Phenol is toxic and corrosive. Always handle phenol-containing reagents in a certified chemical fume hood and wear appropriate personal protective equipment (PPE) (lab coat, gloves, and safety goggles/face shield). Avoid skin or eye contact and avoid inhaling vapors. If contact occurs, follow your institution's safety procedures and the reagent SDS.
- Use disposable, sterile, individually wrapped plasticware and RNase-free pipettes, pipette tips, and tubes.
- Wear disposable gloves when handling reagents and RNA samples to prevent RNase contamination from skin. Change gloves frequently, especially as you move from crude lysates to purified samples.
- Always use good aseptic (RNase-free) technique when working with RNA.
- 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.

Protocol

Sample Preparation

Recommended Sample Input

The magnetic beads in the kit have a defined binding capacity. **Do NOT overload the system.** Exceeding the recommended sample input will overwhelm the beads, leading to reduced RNA recovery, poor purity, and the co-purification of downstream enzymatic inhibitors.

Please strictly adhere to the following input limits and lysis guidelines based on your sample type:

Cultured Cells: RNA abundance varies significantly depending on the cell type and growth conditions. Accurate cell counting prior to extraction is the most reliable method for success.

- **Maximum Input:** Do not exceed **1×10^6 cells**.
- **Starting Recommendation:** If the RNA content of your cell line is unknown, begin with **$\leq 5 \times 10^5$ cells**.
- **Optimization:** You can scale your input up or down in subsequent extractions based on your initial yield and purity results.

Animal Tissue: The maximum allowable tissue input depends on the inherent RNA content of the specific organ. Accurately weighing the tissue before homogenization is critical.

- **General Tissues:** Process up to a maximum of **5 mg**.
- **Adipose (Fat) Tissues:** You may process up to **10 mg**.
- **Quick Reference:** A ~1.5 mm cube of most animal tissue typically weighs approximately 3.5 to 4.5 mg.

Lysis and Homogenization

Use a sufficient volume of Lysis Reagent, such as TRIzol Reagent, to fully lyse and homogenize the sample. Incomplete lysis or homogenization may leave cellular debris in the aqueous phase. This can interfere with magnetic bead binding and reduce RNA recovery and purity.

Sample Preparation - Step 1: Lysis

Select the appropriate protocol below based on your sample type.

⚠***IMPORTANT:***

- **Complete Medium Removal:** Residual culture medium will dilute the lysate, inhibit lysis efficiency, and reduce downstream bead binding and RNA yield.
- **Do Not Wash:** Do not wash cells with PBS or other buffers prior to adding the Lysis Reagent, as this increases the risk of RNA degradation.

A. Cultured Cells

- **Adherent Cells:** Completely aspirate the culture medium. Add 1 mL Lysis Reagent (e.g., TRIzol or TRI Reagent) directly to the dish per 10 cm^2 of growth area (equivalent to one well of a 6-well plate). Pipette up and down repeatedly to completely homogenize. Incubate for 5 min at room temperature.
- **Suspension Cells:** Centrifuge at 300 × g for 5 min to pellet cells. Carefully aspirate all supernatant. Add 50 µL of kit-provided **Resuspension Buffer**, then vortex briefly to fully resuspend the cell pellet. Immediately add 1 mL Lysis Reagent per $5x10^5$ cells. Pipette or vortex to homogenize. Incubate for 5 min at room temperature. *(Note: Tougher strains, such as yeast or bacteria, require mechanical disruption via bead beating).*

B. Exosomes

- **Column Based Exosome Isolation**

Follow the original manufacturer's protocol for exosome isolation, lysis on the column (e.g., exoEasy spin column), phase separation, and collecting the clear upper aqueous phase. The resulting upper aqueous phase can be used directly as your starting input for this kit. Proceed directly to **Step 2: Enrich** in the main procedure.

- **Pellet Forming Exosome Isolation (Ultracentrifugation or PEG)**
  1. Harvest: Obtain the exosome pellet generated by ultracentrifugation or PEG-based precipitation. Carefully discard all supernatant. Briefly spin down again to remove any residual liquid.
  2. Resuspend *(CRITICAL):* Add 50 µL of kit-provided **Resuspension Buffer**. Gently vortex or pipette until fully resuspended. *Do NOT use PBS or proprietary buffers from other exosome kits.*
  3. Lyse: Immediately add 1 mL Lysis Reagent to the resuspension. Vortex vigorously for 15 seconds, then incubate for 5 min at room temperature.

C. Animal Tissue

1. Excise up to **5 mg** of tissue. Do not allow frozen tissue to thaw before adding the lysis buffer. Add 1 mL Lysis Reagent.
2. Immediately disrupt the tissue using a mechanical homogenizer (e.g., rotor-stator or bead beater).
   Note: Vortexing alone is insufficient.
3. Incubate for 5 min at room temperature.
   Optional: If the lysate contains high amounts of insoluble debris, centrifuge at 12,000 x *g for 10 min at 4°C, and transfer the supernatant to a new tube.*

D. Plant Tissue

1. Grind: Grind plant tissue to a fine powder in liquid nitrogen using a mortar and pestle. Keep the sample frozen during grinding.
2. Add PVP (If applicable): For mature leaves or complex tissues rich in polysaccharides/polyphenols (e.g., potato, cotton, woody plants), add solid PVP-40 (1–2% w/v) to the frozen powder. *(Note: This step is not required for young tissues or model plants like Arabidopsis).*
3. Lyse: Add 1 mL Lysis Reagent per 50–100 mg of tissue powder. Vortex vigorously for 30 seconds. Incubate for 5 min at room temperature.

Sample Preparation - Step 2: Phase Separation

1. *(Optional)* For samples with high fat content or substantial insoluble material, centrifuge the lysate at 12,000 × g for 5 min at 4-10°C, then transfer the clear supernatant to a new tube.
2. Add 0.2 mL chloroform per 1 mL of Lysis Reagent used.
3. Cap the tube securely and vortex for 15 seconds.
4. Incubate at room temperature for 2-3 min.
5. Centrifuge at 12,000 × g for 15 min at 4°C. The mixture will separate into a lower red phenol–chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains in the aqueous phase, while DNA and proteins partition into the interphase and organic phase.
   Note: Perform phase-separation centrifugation at 4-10°C. Higher temperatures may increase DNA carryover into the aqueous phase.
6. Carefully transfer the upper aqueous phase to a new tube.
   Note: This aqueous phase contains the RNA and is the input for this kit (see main procedure below).

Procedure

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 **R1 Binding Buffer**: Add 22.5 mL 100% ethanol to 7.5 mL R1 Concentrate (Final volume: 30 mL). Mix well after adding ethanol. Tightly cap the bottle and store at room temperature.
- Prepare **W4 Washing Buffer**: Add 46 mL 100% ethanol to 23 mL W4 Concentrate (Final volume: 69 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:

- Ensure there is no precipitates in R1 and W4. If precipitate is present, warm the buffer at 37°C and mix until completely dissolved.
- Pre-warm Elution Buffer to 60°C.

Step 1: Collect the Upper Aqueous Phase

Process the sample using acid phenol–chloroform reagent, such as TRIzol Reagent or TRI Reagent, according to **Sample Preparation** or manufacturer's instructions. Ensure upper aqueous phase has been transferred to a new 2 mL RNase-free microcentrifuge tube.

- ***Volume Tip****: 1 mL of Lysis Reagent typically yields ~600 µL of aqueous phase. To ensure maximum purity, it is recommended to transfer only the top 400 µL.*
- ⚠***CRITICAL****: Avoid disturbing or transferring any of the white interphase or lower organic layer, as this contains DNA and proteins.*

Step 2: Enrich

Choose either **Section A** or **Section B** based on the desired RNA fraction. Perform **Section A** to enrich small RNA (<100 nt), or perform **Section B** to recover total RNA.

Section A. Enrich Small RNA

2.1 For each 400 µL of aqueous phase collected in Step 1, add 800 µL **R1 Binding buffer** and 20 µL fully resuspended **R2 beads**.

⚠***CRITICAL:*** *Ensure that 100% ethanol has already been added to the R1 Concentrate prior to use (see* ***Preparation Before First Use****).*

2.2 Vortex for 5 min.

2.3 Put the tube on a magnetic stand for 5 min, or until the solution is clear.

2.4 Transfer the supernatant to a new tube. Discard the beads.

2.5 Add 35 µL fully resuspended **R3 beads** to the Step 2.4 supernatant.

2.6 **Flex-Range Selection**: Select the desired RNA recovery mode (Standard Mode or Discovery Mode). Add the appropriate volume of 100% ethanol to the collected supernatant according to the table below.

	100% ethanol
Standard Mode (Recover ≥15 nt)	186 µL
Discovery Mode (Recover >10 nt)	710 µL

***Note**: Use Reverse Pipetting technique when handling ethanol (see Appendix).*

2.7 Vortex for 5 min.

***Note**: Vigorous shaking is critical for efficient binding of small RNA.*

2.8 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.

Section B. Enrich Total RNA

2.1 For each 400 µL of aqueous phase collected in Step 1, add 800 µL **R1 Binding buffer** and 35 µL fully resuspended **R3 beads**.

⚠***CRITICAL:** Ensure that 100% ethanol has already been added to the R1 Concentrate prior to use (see **Preparation Before First Use**).*

2.2 **Flex-Range Selection**: Select the desired RNA recovery mode (Standard Mode or Discovery Mode). Add the appropriate volume of 100% ethanol to the collected supernatant according to the table below.

	100% ethanol
Standard Mode (Recover ≥15 nt)	162 µL
Discovery Mode (Recover >10 nt)	670 µL

***Note**: Use Reverse Pipetting technique when handling ethanol (see Appendix).*

2.3 Vortex for 5 min.

***Note**: Vigorous shaking is critical for efficient binding of small RNA.*

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.

Step 3: Washing

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: Elution

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 25 ~ 50 µL of pre-warmed **Elution Buffer** to the beads, vortex for 5 min.

Note： *For small RNA enrichment (Step 2, Section A), a lower elution volume, such as 25 µL, is recommended to obtain a more concentrated eluate. For total RNA recovery (Step 2, Section B), a higher elution volume, such as 50 µL, is recommended to improve overall RNA recovery.*

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 free microcentrifuge tube. Discard the bead.

Storage: *Keep eluted RNA on ice for immediate use. For long-term storage, store at -80°C.*

Note 1: *The small RNA eluate may contain abundant tRNA. For near-complete depletion of tRNA and other background RNA, further process the eluate using the* ***DeepCatch™ Small Nucleic Acid Fractionation Kit (GM1016, GM1048)****.*

Note 2 (Optional Concentration): *If further concentration is required, the* ***DeepCatch™ Broad-Range Nucleic Acid Concentration Kit (GM1348)*** *can be used to concentrate ~200 µL of eluate to a final volume as low as 5 µL.*

Note 3: *DNase treatment is generally not required, as the combined efficiency of phenol-chloroform phase separation and the DeepCatch™ depletion workflow remove most DNA efficiently without enzymatic digestion. In most applications that measure mature miRNAs, trace amounts of genomic DNA do not interfere with assay performance. However, some RNA workflows are highly sensitive to even very small amounts of DNA. For these applications, an optional DNase digestion may be performed after RNA purification to remove any remaining residual DNA.*

Troubleshooting Guide

1. Phases Do Not Separate Completely

- **Chloroform not added, or incorrect chloroform used**
  Ensure chloroform was added at the correct volume. Use pure chloroform without isoamyl alcohol or other additives.
- **Insufficient mixing after chloroform addition**
  After adding chloroform, the sample must be mixed vigorously. Vortex/shake for ≥15 seconds, then incubate for 2-3 min and centrifuge.
- **Incompatible components in the starting sample**
  Avoid processing samples that contain organic solvents (e.g., ethanol, DMSO) or strong/alkaline buffers, as these can disrupt phase separation. If such reagents were used upstream, remove them or dilute/replace the buffer before TRI Reagent/TRIzol-based extraction.

2. Low Yield

- **Protocol not followed exactly**
  Follow the procedure strictly. Do not change reagent volumes. Keep incubation times and temperatures within the specified ranges, as deviations can significantly reduce yield.
- **Too much starting material**
  Do not exceed recommended input. If yield is low or inconsistent, reduce input (e.g., start with ≤ 5×10^5 cells and do not exceed 1×10^6 cells; use ≤ 5 mg tissue unless otherwise specified).

- **Incomplete disruption or homogenization**
  Ensure the sample is fully disrupted and the lysate is uniform. If needed, increase homogenization time and clarify the lysate by centrifugation (higher g-force and/or longer spin).
- **Missing the 5-minute post-homogenization incubation**
  After homogenization, incubate the lysate at room temperature for 5 min as specified. This step supports complete dissociation of protein from the protein-nucleic acid complexes.
- **Improper sample storage or repeated freeze–thaw**
  Avoid multiple freeze–thaw cycles of samples, which can degrade nucleic acids. Use fresh material when possible and store aliquots appropriately.
- **Ethanol not suitable for binding steps**
  Use 99.5–100% ethanol (Molecular Biology grade or ACS grade). Do not use old, diluted, or denatured ethanol.
- **RNase contamination / RNA degradation**
  Maintain RNase-free technique throughout: wear clean gloves, use RNase-free tubes and aerosol-resistant tips, and work on a clean bench with clean reagents. Keep tubes capped and minimize unnecessary time at room temperature.
- **Excess residual cell-culture medium carried into lysis**
  Residual medium can dilute the lysis reagent and interfere with extraction and phase separation. Remove medium completely (adherent cells: aspirate thoroughly; suspension cells: pellet and discard the supernatant). Avoid carryover of medium into the lysis step.
- **Adherent Cells were rinsed/washed before adding Lysis Reagent**
  Do not rinse/wash adherent cells prior to lysis; add Lysis Reagent directly after removing medium.
- **Cells pellet or exosomes/EV pellet were resuspended in an incorrect buffer**
  Resuspend the pellet in 50 µL of the kit-provided Resuspension Buffer (required) before adding Lysis Reagent.
- **Cell pellet or exosomes/EV pellet was not fully resuspended before lysis**
  After adding the kit-provided Resuspension Buffer to the pellet, make sure the pellet is fully dispersed before adding Lysis Reagent. Flick the bottom of the tube several times or pulse-vortex for a few seconds to loosen the pellet. Complete resuspension helps ensure efficient lysis and improves RNA recovery.
- **Elution performed at low temperature**
  Pre-warm Elution Solution to 60°C. Elution at lower temperature may significantly reduce recovery.

3. Unreliable or Low Concentration Readings

- **Low RNA mass in small / ultra-short RNA fractions**
  Small RNA yields, especially from exosome or low-input samples, are often very low. Concentration readings may therefore be weak, variable, or below the reliable detection range of common quantification methods.
- **Limitations of UV absorbance (A260)**
  UV-based measurements, such as NanoDrop™, are not recommended for low-input small RNA or ultra-short RNA fractions. When RNA mass is low, A260 readings may mainly reflect background absorbance from salts, phenol, buffers, or other carryover components rather than true RNA content.
- **Fluorescence-based assays**
  Fluorescence-based assays, such as Qubit™ RNA assays, are generally more reliable than UV absorbance for low-input RNA. However, standard RNA fluorescence assays are typically optimized for longer RNA molecules and may show reduced or nonlinear signal response to ultra-short RNA fragments, especially in the 10–40 nt range. Therefore, a low or undetectable Qubit™ reading does not necessarily indicate poor recovery of ultra-short RNA.
- **Recommended evaluation**
  For small and ultra-short RNA fractions, assess recovery using methods suitable for short RNA analysis, such as

denaturing PAGE, Bioanalyzer™, TapeStation™, Fragment Analyzer™, RT-qPCR/ddPCR for defined targets, spike-in controls, or small RNA-seq library QC. When quantitative comparison is required, use synthetic RNA standards of similar size whenever possible.

4. RNA Degraded

- **Improper sample handling**
  For frozen tissues, ensure samples were snap-frozen in liquid nitrogen immediately and kept frozen until lysis. Process samples promptly, especially during the early steps.
- **RNase contamination**
  Although kit reagents are RNase-free, RNases can be introduced during handling. Use RNase-free technique (clean gloves, RNase-free consumables, clean work area). Avoid drying RNA in equipment that may be contaminated (e.g., vacuum dryers previously used with reagents or workflows where RNases may have been present).
- **Excess residual cell-culture medium carried into lysis**
  Residual medium can dilute the lysis reagent and interfere with extraction and phase separation. Remove medium completely (adherent cells: aspirate thoroughly; suspension cells: pellet and discard the supernatant). Avoid carryover of medium into the lysis step.

5. DNA Contamination

- **Phase separation performed too warm**
  Perform the phase-separation centrifugation at 4-10°C. Ensure the centrifuge does not warm above 10°C during the spin.
- **Interphase carryover into the aqueous phase**
  DNA contamination commonly occurs when the interphase is disturbed. Carefully transfer only the upper aqueous phase, leaving a small volume behind if needed to avoid interphase carryover.

6. Poor Performance in Downstream Applications (e.g., PCR/NGS Failure)

- **Residual Ethanol:** Ethanol carryover inhibits enzymes. Ensure beads are air-dried (matte appearance) before elution.
- **Over-drying:** Do not dry beads until they crack, as this prevents DNA from eluting efficiently.
- **Salt Contamination:** Ensure the 80% Ethanol wash steps are performed thoroughly.

7. Magnetic Beads Issues

- **Hard to Resuspend:** They may have been over-dried (>10 minutes). Reduce air-drying time.
- **Tight Pellet:** If the magnetic stand is very strong, the pellet may be packed tight. Pipette up and down vigorously or vortex to fully release the beads.

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. Advanced tRNA Depletion Workflow

For applications requiring further depletion of tRNA and other background RNA, purified RNA from this kit can be further processed using the **DeepCatch™ Small Nucleic Acid Fractionation Kit, Mode A**. This combined two-kit approach provides a streamlined end-to-end workflow for targeted enrichment of ultra-short RNA fractions, such as the 10–40 nt RNA fraction.

	DeepCatch™ Small Nucleic Acid Fractionation Kit (Mode A) Cat# GM1016, GM1048
Size Cutoff	**Target Enrichment:** 10-40 nt **Background Depletion:** > 40 nt
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.

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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