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Concentrating RiboMinus™ RNA Using PureLink™ RNA Mini Kit or RiboMinus™ Concentration Module

Introduction

After purifying RiboMinus™ RNA using the RiboMinus™ Eukaryote Kit for RNA-Seq, you need to concentrate RiboMinus™ RNA for further use in downstream applications. To retain all species of RNA <200 nucleotides (nt), you have three options to concentrate RiboMinus™ RNA:

- Ethanol precipitation (for the protocol, see the manual supplied with RiboMinus™ Eukaryote Kit for RNA-Seq)
- PureLink™ RNA Mini Kit using silica spin columns (Cat. no. 12183018A or 12183020), see page 2 for a **modified** protocol
- RiboMinus™ Concentration Module using silica spin columns (Cat. no. K1550-05), see page 3 for a **modified** protocol

Generally, RNA species <200 nt are excluded from the standard binding conditions for RNA isolation using silica spin column. Recent studies have shown the importance of these small RNA species which include regulatory RNA molecules such as microRNA (miRNA), short interfering RNA (siRNA), snRNA, and other RNA transcripts of yet unknown function.

To retain all species of RNA <200 nucleotides (nt) during the concentration step of RiboMinus™ RNA isolation, use the modified silica spin column protocol with PureLink™ RNA Mini Kit or RiboMinus Concentration Module wherein the binding of RNA is performed with 50% ethanol to retain all RNA species including <200 nt.

System Overview

The binding conditions of the spin column protocol are optimized for the RiboMinus™ RNA sample with ethanol and buffer. The sample is loaded onto a spin column. The RiboMinus™ RNA binds to the silica-based membrane in the column and impurities are removed by thorough washing with Wash Buffer. The RiboMinus™ RNA is then eluted in RNase-free water.

Important

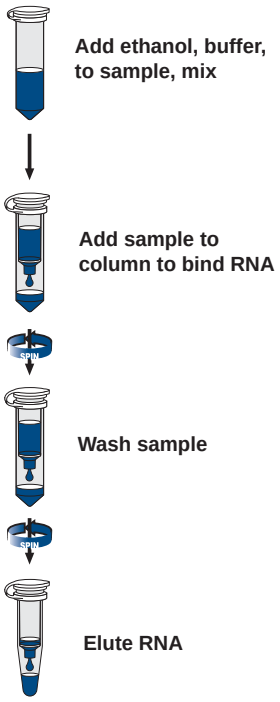
Do not use the purification protocols included in the PureLink™ RNA Mini Kit and RiboMinus™ Concentration Module manuals for concentrating RiboMinus™ RNA as using the standard RNA spin column purification protocols will result in loss of small RNA species. Use the modified spin column purification protocols included in this manual for recovering all small RNA species including <200 nt.

Caution

The PureLink™ RNA Mini Kit and RiboMinus™ Concentration Module buffers contain guanidine isothiocyanate. Always wear a laboratory coat, disposable gloves, and eye protection when handling buffers.

Do not add bleach or acidic solutions directly to solutions containing guanidine isothiocyanate or sample preparation waste as it forms reactive compounds and toxic gases when mixed with bleach or acids. Solutions containing ethanol are considered flammable. Use appropriate precautions when using this chemical. Dispose of the buffers and chemicals in appropriate waste containers.

Workflow



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General Guidelines

- Use disposable, individually wrapped, sterile plasticware and use sterile, new pipette tips and microcentrifuge tubes
- Wear latex gloves while handling reagents and RNA samples to prevent RNase contamination from the skin surface
- Always use proper microbiological aseptic techniques when working with RNA
- Use RNase *AWAY*® Reagent (Cat. no. 10328-011) to remove RNase contamination from surfaces

Using PureLink™ RNA Mini Kit to Concentrate RiboMinus™ RNA

Use the **modified** protocol described below to concentrate RiboMinus™ RNA with PureLink™ RNA Mini Kit to retain all RNA species including <200 nt.

Materials Needed

- RiboMinus™ RNA (obtained using RiboMinus™ Eukaryote Kit for RNA-Seq, Cat. no. A10837-02 or A10837-08)
- PureLink™ RNA Mini Kit (Cat. no. 12183018A or 12183020)
- 96–100% ethanol
- Microcentrifuge capable of centrifuging >12,000 × g

Before Starting

Before using Wash Buffer II for the first time, add 60 ml (Cat. no. 12183018A) or 16 ml (Cat. no. 12183020) 96–100% ethanol directly to the Wash Buffer II bottle. Check the box on the Wash Buffer II label to indicate that ethanol was added. Store Wash Buffer II with ethanol at room temperature.

Modified PureLink™ RNA Mini Kit Purification protocol

1. Transfer the **RiboMinus™ RNA** (~ 530 µl) sample to a new tube capable of holding >2 ml.
2. Add 1X sample volume of Lysis Buffer (530 µl for this protocol) and 96–100% ethanol to a final concentration of 50% (1,060 µl for this protocol). Mix thoroughly by vortexing.

   Note: The binding step is performed with a higher (50%) ethanol concentration than is used for standard protocol to ensure recovery of all RNA species including <200 nt typically not retained using standard binding conditions for silica column RNA purification.
3. Transfer up to 700 µl of the sample (from Step 2) to the Spin Cartridge (with the collection tube).
4. Centrifuge at 12,000 × *g* for 1 minute at room temperature. Discard the flow-through, and reinsert the Spin Cartridge into the same Collection Tube.
5. **Repeat** Steps 3–4 until the entire sample is processed.
6. Wash the column with 500 µl Wash Buffer II prepared with ethanol. Centrifuge the column at 12,000 × g for 1 minute at room temperature. Discard the flow through.
7. Discard the collection tube and place the column into a clean collection tube, supplied with the kit.
8. Centrifuge the column at maximum speed for 2–3 minutes at room temperature to remove any residual Wash Buffer II. Place the column in a clean 1.5-ml Recovery Tube.
9. Add 30–100 µl of RNase-free Water to the center of the column.
10. Incubate the column at room temperature for 1 minute.
11. Centrifuge the column at maximum speed for 1 minute at room temperature.

    The Recovery Tube contains purified RiboMinus™ RNA.
12. Place RiboMinus™ RNA on ice to proceed to the desired downstream application or store RiboMinus™ RNA at –80°C.

Using RiboMinus™ Concentration Module to Concentrate RiboMinus™ RNA

Use the **modified** protocol described below to concentrate RiboMinus™ RNA with RiboMinus™ Concentration Module to retain all RNA species including <200 nt.

Materials Needed

- RiboMinus™ RNA (obtained using RiboMinus™ Eukaryote Kit for RNA-Seq, Cat. no. A10837-02 or A10837-08)
- RiboMinus™ Concentration Module (Cat. no. K1550-05)
- 96–100% ethanol
- Microcentrifuge capable of centrifuging >12,000 × g

Before Starting

Before using Wash Buffer (W5) for the first time, add 6 ml 96-100% ethanol to 1.5 ml Wash Buffer (W5) included with the kit. Check the box on the Wash Buffer label to indicate that ethanol was added. Store Wash Buffer (W5) with ethanol at room temperature.

Modified RiboMinus™ Concentration Module protocol

1. Transfer the **RiboMinus™ RNA** (~ 530 µl) sample to a new tube capable of holding >2 ml.
2. Add 1X sample volume of Binding Buffer L3 (530 µl for this protocol) and 96–100% ethanol to a final concentration of 50% (1,060 µl for this protocol). Mix thoroughly by vortexing.

   Note: The binding step is performed with a higher (50%) ethanol concentration than is used for standard protocol to ensure recovery of all RNA species including <200 nt typically not retained using standard binding conditions for silica column RNA purification.
3. Transfer up to 700 µl of the sample (from Step 2) to the spin column (with the collection tube).
4. Centrifuge at 12,000 × *g* for 1 minute at room temperature. Discard the flow-through, and reinsert the Spin Column into the same Collection Tube.
5. **Repeat** Steps 3–4 until the entire sample is processed.
6. Wash the column with 500 µl Wash Buffer (W5) prepared with ethanol. Centrifuge the column at 12,000 × g for 1 minute at room temperature. Discard the flow through.
7. Discard the collection tube and place the column into a clean collection tube, supplied with the kit.
8. Centrifuge the column at maximum speed for 2–3 minutes at room temperature to remove any residual Wash Buffer (W5). Place the column in a clean, 1.5-ml Recovery Tube.
9. Add 30–100 µl of RNase-free Water to the center of the column.
10. Incubate the column at room temperature for 1 minute.
11. Centrifuge the column at maximum speed for 1 minute at room temperature.

    The Recovery Tube contains purified RiboMinus™ RNA.
12. Place RiboMinus™ RNA on ice to proceed to the desired downstream application or store RiboMinus™ RNA at -80°C.

Analyzing RiboMinus™ RNA

The purified RiboMinus™ RNA is easily quantitated using UV absorbance at 260 nm or Quant-iT™ RNA Assay Kit. The RNA isolated is of high-quality and is up to 99.9% depleted in ribosomal RNA (rRNA) species.

To verify rRNA depletion, perform agarose gel electrophoresis of the sample or use a bioanalyzer. Agarose gel electrophoresis analysis shows depletion of 18S and 28S rRNA bands as compared to a control sample. Absence of contaminating DNA and RNA degradation may also be confirmed by agarose gel electrophoresis. The efficiency of rRNA depletion in RiboMinus™ RNA, RNA degradation, and RNA concentration can also be analyzed using a bioanalyzer such as the Agilent 2100 bioanalyzer with an RNA LabChip®.

Troubleshooting

Problem	Cause	Solution
Low RiboMinus™ RNA yield	Incorrect binding conditions	For efficient binding of RiboMinus™ RNA to the spin column, ensure the sample contains 50% ethanol prior to loading onto the spin column.
	Ethanol not added to Wash Buffer	Be sure to add 96–100% ethanol to Wash Buffer as described on pages 2 and 3.
	Incorrect elution conditions	Add water to the center of the column and perform incubation for 1 minute with water before centrifugation.
	RNA quantitation performed with water	Be sure the RNA quantitation using UV absorbance is performed with 10 mM Tris-HCl, pH 7.0 to accurately measure the UV absorbance.
RNA degraded	RNA contaminated with RNase	Follow the guidelines on page 2 to prevent RNase contamination.
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified RNA sample	Traces of ethanol from the Wash Buffer can inhibit downstream enzymatic reactions.
		To remove any residual Wash Buffer, discard collection tube with Wash Buffer flow through. Reinsert the spin column into a new, collection tube and centrifuge the spin column at maximum speed for 2–3 minutes to completely dry the column.

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