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Low Input RiboMinus™ Eukaryote System v2

Catalog Number A15027

MAN 0007160 **Revision** 2.0

IMPORTANT! Before using this kit, read and understand the information in the "Safety" section of this document.

Product description

The Low Input RiboMinus™ Eukaryote System v2 provides a robust and efficient method for removal of cytoplasmic (5S, 5.8S, 18S and 28S) and mitochondrial (12S and 16S) ribosomal RNA (rRNA) from 100 ng–1 µg of total RNA, in a single round of depletion. The system utilizes probe designs from highly conserved regions of rRNA, which enables use for several eukaryotic species such as human, mouse, and rat. Optimized hybridization conditions enable depletion in less than an hour.

In addition, the Low Input RiboMinus™ Eukaryote System v2 includes a magnetic bead-based RiboMinus™ Magnetic Bead Clean Up Module for concentration and purification of the rRNA-depleted RNA, enabling easier handling and scalability. The Low Input RiboMinus™ Eukaryote System v2 effectively recovers polyA mRNA, non-polyadenylated RNA, pre-processed RNA, tRNA, and RNA transcripts less than 200 nt. The resulting rRNA-depleted RNA is suitable for whole transcriptome analysis by RNA-Seq or other downstream applications.

Kit contents

Each system or kit supplies reagents sufficient for 12 reactions using 100 ng–1 µg of total RNA. Reagents are shipped at 4°C; refer to the following table for long-term storage information.

Component	Cap color	Volume	Storage
Low Input RiboMinus™ Eukaryote Oligo Module v2			
RiboMinus™ Eukaryote Probe Mix v2	Clear	30 µL	–20°C
Low Input RiboMinus™ Core Module v2			
2X Hybridization Buffer	Yellow	1.5 mL	Room temp.
RiboMinus™ Magnetic Beads	Blue	3 mL	4°C
RiboMinus™ Magnetic Bead Clean Up Module			
Binding Solution Concentrate	Clear	6 mL	Room temp.
Nucleic Acid Binding Beads	White	0.15 mL	4°C
Wash Solution Concentrate (add 8mL of 100% Ethanol before use)	Clear	2 mL	Room temp.

Required materials not provided

Material	Source†
DynaMag™ 2 Magnetic Stand or equivalent	Cat. no. 123-21D
Heat blocks or water baths set to 37°C, 50°C, and 70°C	MLS
Nuclease-free Water	Cat. no. AM9938
Ethanol, 100% ACS reagent grade or equivalent	MLS
Microcentrifuge	MLS
Pipettors	MLS
Pipet tips, RNase-free	MLS
Non-stick RNase-free Microfuge Tubes, 1.5 mL	Cat. no. AM12450

† **www.lifetechnologies.com** unless otherwise indicated. MLS: major laboratory supplier.

Procedure overview

The workflow is shown on page 3. First, total RNA is hybridized with biotinylated RiboMinus™ Eukaryote Probe Mix v2. Next, the rRNA-probe complexes are removed from the total RNA by capture with streptavidin-conjugated RiboMinus™ Magnetic Beads. The resulting rRNA-depleted RNA is concentrated and purified with Nucleic Acid Binding Beads.

Procedural guidelines

Input RNA

Each reaction uses 100 ng–1 µg of total RNA. We recommend isolating total RNA using the PureLink® RNA Mini Kit (Cat. no. 12183018A or 12183020), TRIzol® Reagent (Cat. no. 15596-026), or the MagMAX™ FFPE Total Nucleic Acid Isolation Kit (Cat. no. 4463365). If your downstream application requires DNA-free RNA, perform DNase treatment of the total RNA before performing the RiboMinus™ procedure. Check the quality of your total RNA, including DNA contamination.

General handling of RNA

- Use disposable, individually wrapped, sterile plasticware and use sterile, new pipette tips and non-stick 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*Zap*® decontamination solution (Cat. no. AM9780, AM9786) to remove RNases from work surfaces.

Hybridize RiboMinus™ Eukaryote Probe Mix v2 and total RNA sample (~30 minutes)

Start with 100 ng–1 µg of total RNA

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Hybridize with RiboMinus™ Eukaryote Probe Mix v2 (70°C ▸ 37°C)

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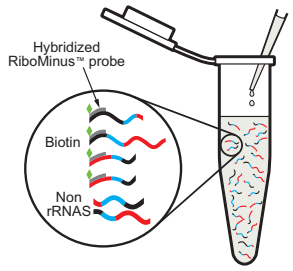

Capture and remove rRNA-probe complexes (~6 minutes)

Bind to prepared RiboMinus™ Magnetic Beads

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Magnetically capture and remove beads

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Keep the supernatant—contains the rRNA-depleted RNA

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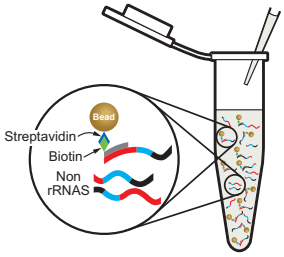

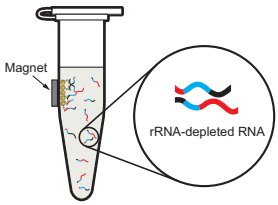

Concentrate the rRNA-depleted RNA (~25 minutes)

Bind the supernatant to Nucleic Acid Binding Beads

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Magnetically capture the beads

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Wash the beads with prepared Wash Solution

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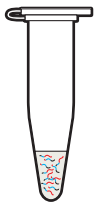
Elute the rRNA-depleted RNA in 70°C Nuclease-free Water



RiboMinus™ procedure

Hybridize RiboMinus™ Eukaryote Probe Mix v2 and total RNA sample

Before you begin:

- Pre-heat 2X Hybridization Buffer in a 50°C heat block or bath, to bring salts into solution.
- Set heat blocks to 37°C and 70°C. Arrange heat blocks in close proximity to each other for optimal transfer and slow cooling in step 3.

1. Add the following components to a sterile, RNase-free 1.5-mL microcentrifuge tube in the order listed:

Component	Volume
2X Hybridization Buffer	30 µL
RiboMinus™ Eukaryote Probe Mix v2	2 µL
Total RNA, 100 ng–1µg	X µL
Nuclease-free Water	to 60 µL

2. Mix by gentle vortexing, and incubate the tube with the RNA/probe mix sample (60 µL) at 70°C for 10 minutes to denature the RNA.
3. Immediately transfer the tube to a 37°C heat block, and allow the RNA/probe mix sample to cool to 37°C over a period of 20 minutes.

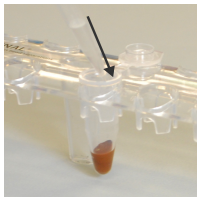
IMPORTANT! Do not allow the sample to cool quickly by excessive time at room temperature during transfer to 37°C or by placing the tubes on ice. Slow cooling promotes sequence-specific hybridization.

While the sample is cooling, prepare the RiboMinus™ Magnetic Beads as described in the following section.

Prepare RiboMinus™ Magnetic Beads

1. Resuspend the RiboMinus™ Magnetic Beads in its bottle (blue cap) by thorough vortexing.

2. For each RNA sample, prepare 140 µL of 1X Hybridization buffer by diluting 2X Hybridization Buffer with an equal volume of Nuclease free Water.
3. For each sample, pipet 250 µL of bead suspension into a sterile, RNase-free, 1.5-mL microcentrifuge tube.
4. Place each tube with the bead suspension on a magnetic stand for 1 minute or until the solution clears. Gently aspirate and discard the supernatant without disturbing the beads.
5. Remove the tubes from the magnetic stand, and wash the beads with 250 µL Nuclease free Water by dispensing the water down the side of the tube where the beads are collected.



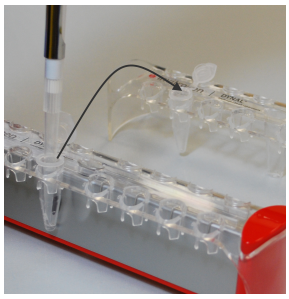
6. Place each tube on a magnetic stand for 1 minute or until the solution clears. Gently aspirate and discard the supernatant without disturbing the beads.
7. Repeat steps 5–6 once.

8. Resuspend the beads in 140 µL of prepared 1X Hybridization Buffer. Place the prepared beads in a 37°C heat block for at least 5 minutes, or longer until the 20-minute incubation of the RNA/probe mix at 37°C is complete.

Capture and remove rRNA-probe complexes

1. After the 20-minute incubation of the RNA/probe mix at 37°C is complete, briefly centrifuge the RNA/probe mix to collect the mixture at the bottom of the tube.
2. Transfer the RNA/probe mix (60 µL) to the prepared RiboMinus™ Magnetic Beads (140 µL). Mix well by pipetting up and down or by low speed vortexing.
3. Place the tube in a 37°C heat block or bath, and incubate for 5 minutes.
4. Briefly centrifuge the tube and place it on a magnetic stand for 1 minute or until the solution clears.
5. Transfer the supernatant (~200 µL) containing the rRNA-depleted RNA to a new tube.

   IMPORTANT! Do not discard the supernatant—this contains the rRNA-depleted RNA.



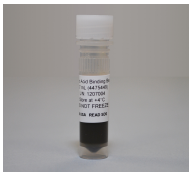
Concentrate the rRNA-depleted RNA

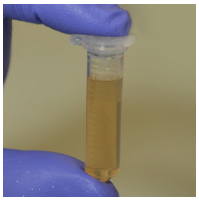
Before you begin:

- Prepare Wash Solution: add 8 mL of 100% ethanol to the bottle of Wash Solution Concentrate and mix well.
- Heat at least 12 µL of Nuclease-free Water per sample to 70°C.

1. In a sterile, RNase-free 1.5-ml microcentrifuge tube, add the following in the order listed and mix when indicated:

Component	Volume
Nucleic Acid Binding Beads (white cap)	10 µL
Binding Solution Concentrate	268 µL
Mix thoroughly by pipetting up and down	
rRNA-depleted RNA (entire eluate)	200 µL
Mix thoroughly by pipetting up and down	
100% Ethanol	668 µL
Ensure the cap is securely closed on the tube, then mix well by inverting the tube. The mixture should look homogeneous.	

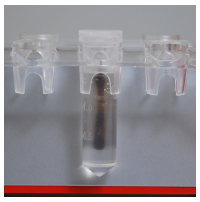




2. Incubate at room temperature for 5 minutes.

   If any sample is retained in the cap, centrifuge the tube briefly to collect the contents at the bottom before proceeding.

3. Place the tube on a magnetic stand for 3 minutes or until the solution clears. Aspirate and discard the supernatant without disturbing the beads.



4. Remove the tube from the stand, and wash the beads by dispensing 300 µL of prepared Wash Solution down the side of the tube where the beads are collected.
5. Replace the tube on the magnetic stand, and allow the solution to clear. Aspirate and discard the supernatant. Carefully remove any remaining supernatant with a 20-µL pipettor without disturbing the bead pellet.
6. Keep the tube on magnetic stand for 2 minutes to allow the beads to air dry.
7. Remove the tube from the stand, and add 12 µL of pre-heated (70°C) Nuclease-free Water. Incubate for 1 minute at room temperature, then place the tube on the magnetic stand.
8. Carefully remove the supernatant containing the eluted rRNA-depleted RNA to a new microcentrifuge tube.

Downstream applications

Typically, ~6% of the input total RNA is recovered after the procedure. For low inputs, we recommend using all the rRNA-depleted RNA for your downstream applications.

Troubleshooting

Observation	Potential cause	Suggested action
Incomplete removal of rRNA	Too much total RNA used	For input RNA amounts ≥1 µg, use the RiboMinus™ Eukaryote System v2 (Cat. no. A15026).
	Improper handling or drying of RiboMinus™ Magnetic Beads	Follow the recommended guidelines for washing and mixing RiboMinus™ Magnetic Beads. Do not allow the beads to dry out after aspirating the supernatant, because drying reduces the bead efficiency.
RNA degraded	RNase contamination	Follow good handling practices to prevent RNase contamination.
	Poor quality starting materials	Always use fresh samples or samples frozen at –80ºC for total RNA isolation. Be sure to check the quality of your total RNA prior to use.
Genomic DNA contamination	Total RNA contained genomic DNA	Treat the total RNA sample with DNase I to remove any genomic DNA contamination before the RiboMinus™ procedure.

Appendix A: Safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).

Specific chemical handling

CAS	Chemical	Notes
26628-22-8	Sodium Azide	Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.
593-84-0	Guanidine Isothiocyanate	Contact with acids or bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid wastes containing this product.

Documentation and support

Visit **www.lifetechnologies.com/support** for product documentation, including Certificates of Analysis and Material Safety Data Sheets, and for product support.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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