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RiboMinus™ Eukaryote System v2
RiboMinus™ Eukaryote Kit v2

Catalog Numbers A15020, A15026

MAN 0007159 **Revision** 4.0

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

Product description

The 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 1–5 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.

The RiboMinus™ Eukaryote System v2 includes a magnetic bead-based RiboMinus™ Magnetic Bead Clean Up Module for concentration of the rRNA-depleted RNA, enabling easier handling and scalability.

The RiboMinus™ Eukaryote Kit v2 contains the rRNA-depletion components only. For concentration of the rRNA-depleted RNA, use the RiboMinus™ Concentration Module (Cat. no. K1550-05), a silica spin column concentration method.

Both bead-based and silica spin column methods effectively recover poly(A) 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 1–5 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
		A15026 (System)	A15020 (Kit)	
RiboMinus™ Eukaryote Oligo Module v2				
RiboMinus™ Eukaryote Probe Mix v2	Clear	60 µL	60 µL	–20°C
RiboMinus™ Core Module v2				
2X Hybridization Buffer	Yellow	2 X 1.6 mL	2 X 1.6 mL	Room temp.
RiboMinus™ Magnetic Beads	Blue	2 X 3 mL	2 X 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 8 mL 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
RNase-free Microfuge Tubes, 2.0 mL	Cat. no. AM12425
Agilent 2100 Bioanalyzer	Agilent – G2938A
Qubit® 2.0 Fluorometer	Cat. no. Q32866
RNA 6000 Pico Kit	Agilent – 5067-1513
Qubit® RNA Assay Kit, 100 assays	Cat. no. Q32852
Required for use with RiboMinus™ Eukaryote Kit v2 (Cat. no. A15020)	
RiboMinus™ Concentration Module	Cat. no. K1550-05
– or –	
Glycogen, 20 µg/µL	Cat. no. 10814-010
3 M sodium acetate in RNase-free water	MLS
96–100% cold ethanol and 70% cold ethanol	MLS

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

Procedure overview

The workflow is shown on page 4. 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. For optimum speed, the resulting rRNA-depleted RNA is concentrated and purified with Nucleic Acid Binding Beads, provided with the RiboMinus™ Eukaryote System v2 (Cat. no. A15026). If the RiboMinus™ Eukaryote Kit v2 (Cat no. A15020) is used, the rRNA-depleted RNA can be concentrated with the RiboMinus™ Concentration Module (Cat. no. K1550-05) or ethanol precipitation.

Procedural guidelines

Input RNA

Each reaction uses 1–5 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™ kit 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 1–5 µg of total RNA

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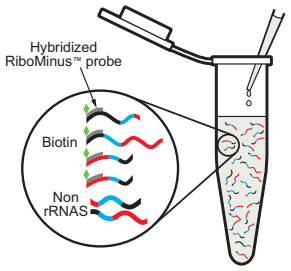
Hybridize with RiboMinus™ Eukaryote Probe Mix v2 (70°C ▸ 37°C)

▼



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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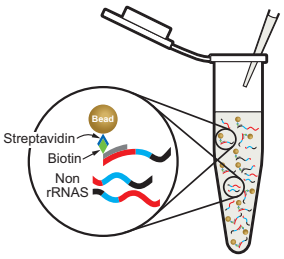
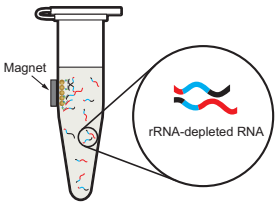
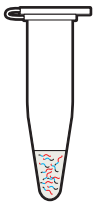




Concentrate the rRNA-depleted RNA

RiboMinus™ Eukaryote System v2 **(~25 minutes)**	RiboMinus™ Eukaryote Kit v2
Bind the supernatant to Nucleic Acid Binding Beads ▼ Magnetically capture the beads ▼ Wash the beads with prepared Wash Solution ▼ Elute the rRNA-depleted RNA in 70°C Nuclease-free Water	Use the RiboMinus™ Concentration Module (Cat. No. K1550-05) *– or –* Ethanol precipitate the RNA



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	50 µL
RiboMinus™ Eukaryote Probe Mix v2	4 µL
Total RNA, 1–5 µg	X µL
Nuclease-free Water	to 100 µL

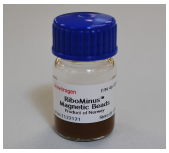
2. Mix by gentle vortexing, and incubate the tube with the RNA/probe mix sample (100 µL) at 70°C for 10 minutes to denature the RNA.
3. Immediately transfer the tube to a 37°C heat block, and allow the RNA/probe mix sample to cool to 37°C over a period of 20 minutes.

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.

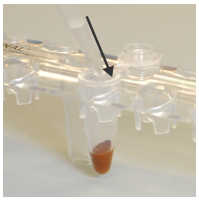
While the sample is cooling, prepare the RiboMinus™ Magnetic Beads as described in the following section.

Prepare RiboMinus™ Magnetic Beads

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



2. For each RNA sample, prepare 200 µL of 1X Hybridization buffer by diluting 2X Hybridization Buffer with an equal volume of Nuclease free Water.
3. For each sample, pipet 500 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 500 µL Nuclease free Water by dispensing the water down the side of the tube where the beads are collected.

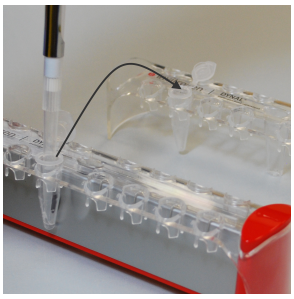


6. Place each tube on a magnetic stand for 1 minute or until the solution clears. Gently aspirate and discard the supernatant without disturbing the beads.
7. Repeat steps 5–6 once.

8. Resuspend the beads in 200 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 (100 µL) to the prepared RiboMinus™ Magnetic Beads (200 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 (~300 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 (RiboMinus™ Eukaryote System v2)

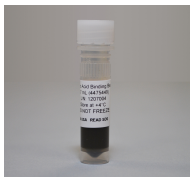
If you are using the RiboMinus™ Eukaryote System v2, follow these instructions to use the included RiboMinus™ Magnetic Bead Clean Up Module to concentrate the rRNA-depleted RNA. If you are using the RiboMinus™ Eukaryote Kit v2, follow the instructions in the next section.

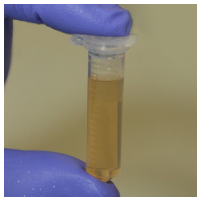
Before you begin:

- 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 2-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	400 µL
Mix thoroughly by pipetting up and down	
rRNA-depleted RNA (entire eluate)	300 µL
Mix thoroughly by pipetting up and down	
100% Ethanol	1000 µL
Ensure the cap is securely closed on the tube, then mix well by inverting the tube. The mixture should look homogeneous.	

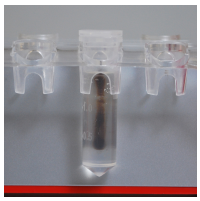




2. Incubate at room temperature for 5 minutes.

   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.

Concentrate the rRNA-depleted RNA (RiboMinus™ Eukaryote Kit v2)

If you are using the RiboMinus™ Eukaryote Kit v2, concentrate the rRNA-depleted RNA using one of the following methods:

- Use the RiboMinus™ Concentration Module (Cat. no. K1550-05).

  IMPORTANT! Follow the *Modified RiboMinus™ Concentration Module Protocol*, available at the web catalog page for the RiboMinus™ Concentration Module, and use **standard-input volumes** to ensure recovery of RNA species <200 nt in length.

- Perform an ethanol precipitation of the RNA:

  a. In a sterile, RNase-free 1.5-mL or 2-mL microcentrifuge tube, add the following components to the supernatant (~300 µL) from "Capture and remove rRNA-probe complexes":

     1 µL glycogen (20 µg/µL)

     30 µL of 3 M sodium acetate

     750 µL of 100% ethanol

  b. Mix well and incubate at –80°C for ≥30 minutes.

  c. Centrifuge the tube for 15 minutes at ≥12,000 × *g* at 4°C. Carefully discard the supernatant without disturbing the pellet.

  d. Add 500 µL of cold 70% ethanol.

  e. Centrifuge the tube for 5 minutes at ≥12,000 × *g* at 4°C. Carefully discard the supernatant without disturbing the pellet.

  f. Repeat steps e–f once.

  g. Air-dry the pellet for ~5 minutes. Resuspend the RNA pellet in 12 µL of Nuclease-free Water.

Analysis of the rRNA-depleted RNA

Typically, ~6% of the input total RNA is recovered after the procedure.

Quantifying the RNA

Assay 1 µL of the undiluted rRNA-depleted RNA using the Qubit® RNA Assay Kit and the Qubit® 2.0 Fluorometer.

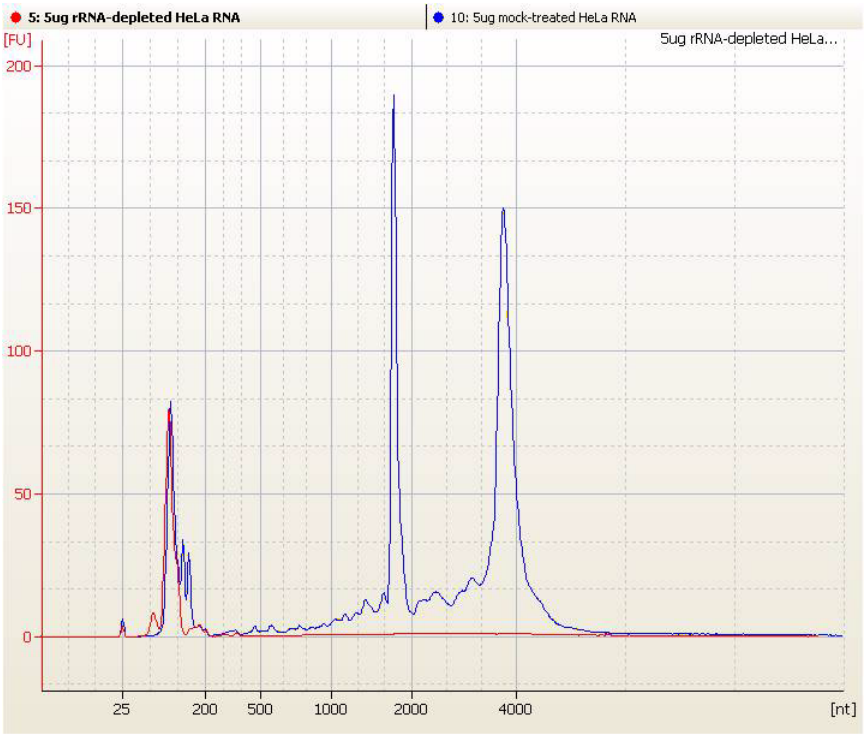
Verifying rRNA depletion

Verify rRNA depletion using one of these methods:

- The Agilent 2100 Bioanalyzer with the Agilent RNA 6000 Pico Kit. This method analyzes the efficiency of rRNA depletion, RNA degradation, and RNA concentration.

  Dilute 1 µL of rRNA-depleted RNA to within the detection range of the RNA 6000 Pico Kit (50–5000 pg/µL), and run 1 µL of the diluted RNA on the Bioanalyzer. See Figure 1.
- Agarose gel electrophoresis. This method can also show depletion of 18S and 28S rRNA bands compared to a control sample.

Figure 1 Example Agilent® Bioanalyzer® instrument analysis of rRNA-depleted RNA (red trace) and mock-treated control total RNA (blue trace). 5 µg of HeLa total RNA was processed using the RiboMinus™ v2 procedure. A mock-treated control sample was processed using the RiboMinus™ v2procedure without RiboMinus™ Eukaryote Probe Mix v2. 1 µL of a 1:10 dilution of each sample was run on the Bioanalyzer® instrument with the RNA 6000 Pico Kit. An overlay of the Bioanalyzer® instrument traces is shown.



Troubleshooting

Observation	Potential cause	Suggested action
Low RNA yield	Low RNA content	Use the Low Input RiboMinus™ Eukaryote System v2 for 100 ng up to1 µg of total RNA.
Incomplete removal of rRNA	Too much total RNA used	For input total RNA amounts >5 µg, divide the input total RNA into two equally sized samples of <5 µg each and run two reactions. Alternatively, use 8 µL of RiboMinus™ Eukaryote Probe Mix v2 to hybridize with the input total RNA. However, this will reduce the number of reactions that can be performed with the kit or system.
	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 Safety Data Sheets (SDSs), and for product support.

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

Document revision history	
Rev. 4.0	Corrected glycogen volume on page 7.
Rev. 3.0	New component volumes in the RiboMinus Magnetic Bead Clean-up Module.
Rev. 2.0	Corrected product catalog number.
Rev. 1.0	Original release.

Limited product warranty

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