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RiboMinus™ Bacteria 2.0 Transcriptome Isolation Kit

USER GUIDE

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For Research Use Only. Not for use in diagnostic procedures.

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Contents

Product information

Product description

The RiboMinus™ Bacteria 2.0 Transcriptome Isolation Kit provides a robust and efficient method for removal of 16S, 23S and, 5S ribosomal RNA from 100 ng to 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 prokaryote species.

The RiboMinus™ Bacteria 2.0 kit includes a RiboMinus™ Magnetic Bead Clean Up Module for concentration of the rRNA-depleted RNA, enabling easier handling and scalability.

The resulting rRNA-depleted RNA is suitable for whole transcriptome analysis by RNA-Seq or other downstream applications.

Contents and storage

Each system or kit supplies reagents sufficient for 12 reactions using 100 ng to 5 µg of total RNA. The RiboMinus™ Core Module and RiboMinus™ Magnetic Bead Clean Up Module are shipped at 4°C, while the RiboMinus™ Pan-Prokaryote Probe Mix is shipped on dry ice. See the following table for long-term storage information.

Component	Cap color	Volume	Storage
RiboMinus™ Pan-Prokaryote Probe Mix [1]	Green	40 µL	–20°C
RiboMinus™ Core Module v2			
2X Hybridization Buffer	Yellow	2 × 1.6 mL	Room temperature
RiboMinus™ Magnetic Beads	Blue	2 × 3 mL	4°C
RiboMinus™ Magnetic Bead Clean Up Module			
Binding Solution Concentrate	Clear	6 mL	Room temperature
Nucleic Acid Binding Beads	Clear	150 µL	4°C
Wash Solution Concentrate (add 8 mL of 100% ethanol before use)	Clear	2 mL	Room temperature

[1] Also available as a standalone product, see “Accessory products” on page 16.

Required materials

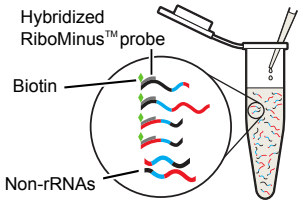
Unless otherwise indicated, materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier..

Item description	Cat. No.
DynaMag™-2 Magnet	12321D
Nuclease-free Water	AM9938
100% ethanol (ACS reagent grade or equivalent)	MLS
Heat blocks, incubators, or thermal cycler set to 37ºC, 50°C, and 70°C	MLS
Microcentrifuge	MLS
Pipettors	MLS
Pipet tips, RNase-free	MLS
Non-stick RNase free Microentrifuge Tubes (1.5-mL and 2-mL)	MLS
RNase-free Microfuge Tubes (2.0 mL)	AM12425

Procedural overview

First, total RNA is hybridized with biotinylated RiboMinus™ Pan-Prokaryote Probe Mix. 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™ Bacteria 2.0 kit.

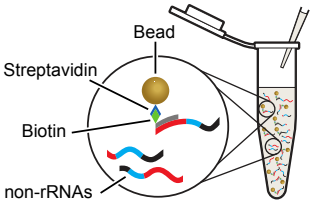
Workflow



"Hybridize probe mix and total RNA"

10 minutes denature

20 minutes hybridization

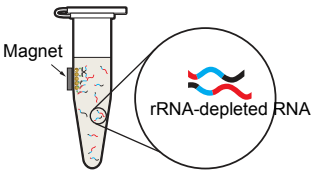


15 minute capture

"Prepare RiboMinus™ Magnetic Beads"

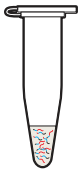
and

"Capture and remove rRNA-probe complexes"



1 minute remove

"Concentrate rRNA-depleted RNA"



25 minute bead clean-up

Methods

Guidelines for RNA samples

Each reaction uses 100 ng to 5 µg of total RNA. Isolate total RNA using the PureLink™ RNA Mini Kit, MagMAX™ *mir*Vana™ Total RNA Isolation Kit, MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit, or TRIzol™ Reagent (see "Accessory products" on page 16). Perform DNase treatment of the total RNA before performing the RiboMinus™ isolation procedure. Check the quality of your total RNA, including DNA contamination.

Guidelines for handling 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*™ RNase Decontamination Solution to remove RNases from work surfaces.

Protocol for depletion of ribosomal RNA from total RNA samples

This protocol describes how to deplete of ribosomal RNA from 100–5,000 ng of total RNA samples using the RiboMinus™ Pan-Prokaryote Probe Mix with modules from the RiboMinus™ Bacteria 2.0 kit.

Prepare 1X Hybridization Buffer

1. Pre-heat 2X Hybridization Buffer at 50°C for at least 5–10 minutes to bring any precipitated salts into solution.
2. Prepare 200 µL of 1X Hybridization Buffer for each RNA sample by diluting 2X Hybridization Buffer with an equal volume of nuclease-free water.

Hybridize probe mix and total RNA

1. Mix the components in the order listed in the following table in a sterile PCR strip tube or 96-well plate.

Component	Volume
2X Hybridization Buffer (yellow cap)	50 µL
RiboMinus™ Pan-Prokaryote Probe Mix (green cap)	1 or 3 µL [1]
Total RNA (100–5,000 ng)	*x* µL
Nuclease-free water	up to 100 µL

[1] Use 1 µL of RiboMinus™ Pan-Prokaryote Probe Mix for samples with 100 ng to <1,000 ng total RNA input, or 3 µL for samples with 1,000–5,000 ng total RNA input.

2. Mix by gentle vortexing, then centrifuge briefly.

3. Incubate the plate/strip in a thermal cycler using the following conditions.

   Note: Set ramp rate at 100%, and heated lid to default at 105°C.

Step	Temperature	Time
Denaturation	10 minutes	70°C
Hybridization	20 minutes	37°C

   Note: Do not place samples in cold water. Do not allow samples to cool to room temperature.

4. During incubation for hybridization, proceed to “Prepare RiboMinus™ Magnetic Beads”.

After the hybridization step is complete, leave the reactions at 37°C, until ready to add sample to prepared RiboMinus™ Magnetic Beads.

Prepare RiboMinus™ Magnetic Beads

1. Resuspend the bottle (blue cap) of RiboMinus™ Magnetic Beads thoroughly by vortexing.
2. For each sample, add 500 µL of bead suspension into a sterile, RNase-free 1.5-mL microcentrifuge tube.
3. Place the tubes 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.
4. Remove the tubes from magnetic stand.
5. Wash the beads with 500 µL nuclease-free water by dispensing the water down the side of the tube where the beads are collected. Alternatively, mix by gentle vortexing, then centrifuge briefly.
6. Place the tubes 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 5–7 one more time.
8. Resuspend the beads in 200 µL of 1X Hybridization Buffer.
9. Place the prepared beads in a 37°C heat block for at least 5 minutes. The beads can stay at 37°C until ready to use with the hybridized RNA/probe mix.

Capture and remove rRNA-probe complexes

1. Briefly centrifuge the RNA/probe mix in the PCR plate/strip tube to collect the contents at the bottom.
2. Transfer each RNA/probe mix (100 µL) sample to a tube containing prepared RiboMinus™ Magnetic Beads (200 µL). Mix well by pipetting or by low speed vortexing.
3. Incubate tubes at 37°C for 15 minutes using a heat block or incubator.
4. Centrifuge the tubes briefly, then place the tubes 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.

Concentrate rRNA-depleted RNA

1. Mix the components in the order listed in the following table in a sterile , RNase-free 2-mL microcentrifuge tube.

Component	Volume
Nucleic Acid Binding Beads (clear cap)	10 µL
Binding Solution Concentrate	400 µL

2. Mix thoroughly by gentle vortexing, or pipetting up and down.

3. Add the entire volume (300 µL) of bead supernatant containing the rRNA-depleted RNA to the microcentrifuge tube, then mix thoroughly by gentle vortexing, or pipetting up and down.

4. Add 1 mL of 100% ethanol to the microcentrifuge tube. Ensure the cap is securely closed, then mix well by inverting the tube.

   Note: The mixture should look homogeneous at this point.

5. 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.

6. Place tube onto a magnetic stand for 3–5 minutes or until the solution clears. Aspirate and discard the supernatant without disturbing the beads.

7. 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. Alternatively, mix by gentle vortexing, then centrifuge briefly.

8. Replace the tube on the magnetic stand, allow solution to clear. Aspirate and discard the supernatant. Carefully remove any remaining supernatant with a 20-µL pipettor without disturbing the bead pellet.

9. Keep the tube on magnetic stand for 2 minutes to allow the beads to air dry, then remove the tube from the stand.

10. Add 12–50 µL of pre-heated (70°C) nuclease-free water to the beads and incubate for 1 minute at room temperature to elute the RNA.

    Note: Use an elution volume suitable for your downstream application.

11. Place the tube on the magnetic stand and carefully collect the supernatant in a new microcentrifuge tube.

    IMPORTANT! The supernatant contains the eluted rRNA-depleted RNA (RiboMinus™ RNA).

Analysis of rRNA-depleted RNA (RiboMinus™ RNA)

- The purified RiboMinus™ RNA is easily quantitated using UV absorbance at 260 nm or with the Qubit™ Fluorometric Quantitation Platform along with the Qubit™ RNA Assay Kit. Expect 3–6% recovery of your total RNA starting input.
- To verify the rRNA depletion, use a Bioanalyzer™ assay or perform agarose gel electrophoresis on the sample. The efficiency for RNA depletion in RiboMinus™ RNA, RNA degradation, and RNA concentration can be effectively analyzed using the Agilent™ 2100 Bioanalyzer™ with the Agilent™ RNA 6000 Pico Kit or the RNA 6000 Nano Kit (see Figure 1, Figure 2).
- Analysis for low-input applications is optional (these are samples starting with 100 ng to <1000 ng input of total RNA into the RiboMinus™ protocol). Where materials are limiting for low input applications, it is recommended that all the RiboMinus™ RNA be used for your downstream application.

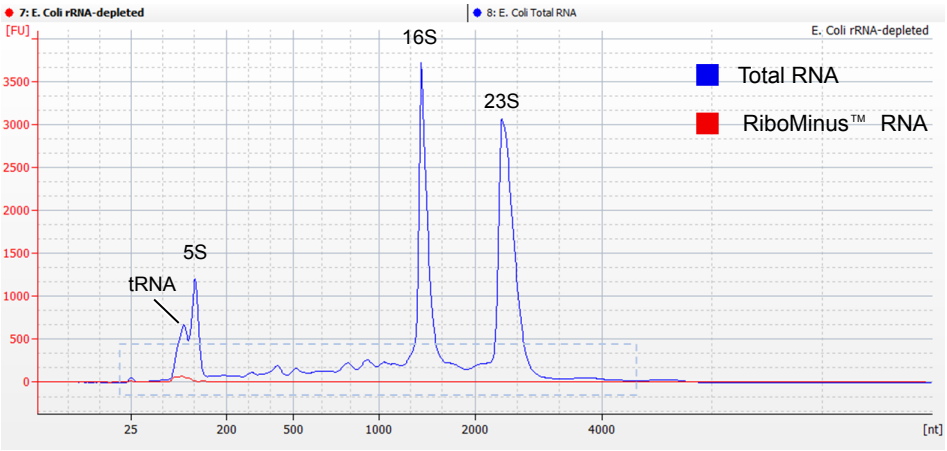


Figure 1 Agilent™ RNA 6000 Pico Assay shows an overlay of RiboMinus™ RNA with *E. coli* total RNA. The RiboMinus™ RNA sample was diluted 1:5 and 1 µL was ran along with 1 µL of a 1:5 dilution of *E. coli* total RNA Samples. 5 µg total RNA input into the depletion reaction.

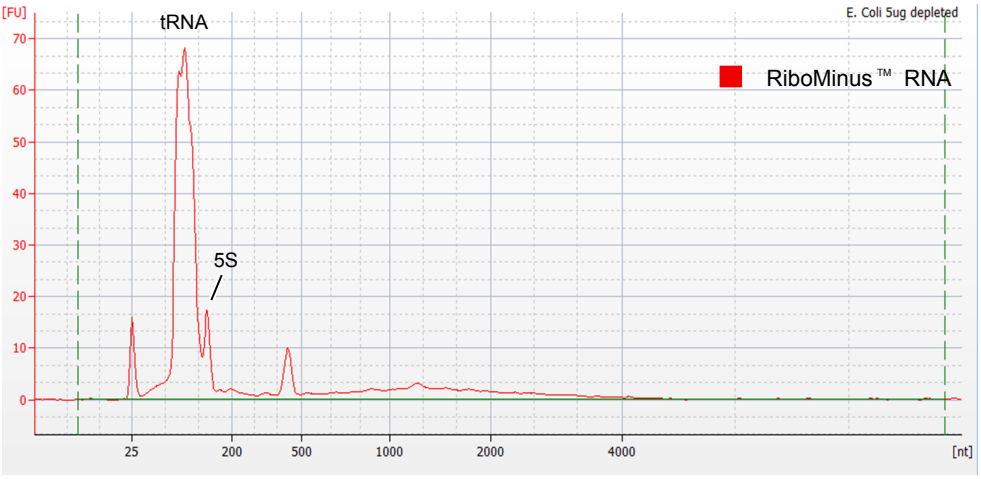


Figure 2 Agilent™ RNA 6000 Pico Assay detailed trace of RiboMinus™ RNA shows absence of rRNA peaks.

Troubleshooting

Observation	Possible cause	Recommended action
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.
	Improper handling or drying of 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 is degraded	The RNA is contaminated by RNase.	Follow good handling practices to prevent RNase contamination.
	The starting materials are of poor quality.	Use fresh samples or samples frozen at –80°C for total RNA isolation. For lysis, process the sample quickly to avoid degradation. 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™ isolation procedure.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical 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. 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).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Related products

Accessory products

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Amount	Cat. No.
RiboMinus™ Pan-Prokaryote Probe Mix	12 reactions	A46920
PureLink™ RNA Mini Kit	10 preps 50 preps	12183020 12183018A
TRIzol™ Plus RNA Purification Kit		12183555
MagMAX™ *mir*Vana™ Total RNA Isolation Kit	96 reactions	A27828
MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit, with bead plates	100 preps	A42357
MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit, with bead tubes	100 preps	A42358
TRIzol™ Reagent	100 mL	15596026
RNase*Zap*™ RNase Decontamination Solution	250 mL	AM9780
RNase*Zap*™ RNase Decontamination Wipes	100 sheets	AM9786
Qubit™ Fluorometer	1 unit	Q32866, Q33216, Q33226
Qubit™ RNA HS Assay Kit		Q32852
Agilent™ 2100 Bioanalyzer™		Agilent G2938A
RNA 6000 Pico Kit		Agilent 5067-1513

Documentation and support

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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the 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 at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.