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RiboMinus™ Bacteria 2.0 Transcriptome Isolation Kit

USER GUIDE

for Automation

for use with KingFisher™ Apex Purification System, KingFisher™ Flex Magnetic Particle Processor, and KingFisher™ Duo Prime Magnetic Particle Processor

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

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Life Technologies Corporation | 2130 Woodward Street | Austin, TX 78744
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.

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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 resulting rRNA-depleted RNA is suitable for downstream applications, such as whole transcriptome analysis by RNA-Seq, genotyping, and qPCR. This protocol guides through automated depletion of ribosomal RNA using the KingFisher™ Apex Purification System, the KingFisher™ Flex Magnetic Particle Processor, or the KingFisher™ Duo Prime Magnetic Particle Processor.

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 20.

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.
Magnetic particle processor (one of the following, depending on quantity/volume of sample to be processed)	
KingFisher™ Apex Purification System with 96 Deep-Well Head	5400930
KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630
KingFisher™ Duo Prime Magnetic Particle Processor	5400110
Equipment and consumables	
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
KingFisher™ Deepwell 96 Plate	95040450
KingFisher™ 96 tip comb for DW magnets (KingFisher™ Apex and KingFisher™ Flex workflow)	97002534
KingFisher™ Duo Prime12-tip comb for Microtiter 96 Deepwell plate (KingFisher™ Duo workflow)	97003500
Elution Strip (KingFisher™ Duo workflow)	97003520
KingFisher™ Duo Cap for Elution Strip (KingFisher™ Duo workflow)	97003540
Conical tubes (15 mL)	AM12500
Conical tubes (50 mL)	AM12501
Reagent reservoirs	MLS
Nuclease-free Water	AM9938
100% isopropanol (ACS reagent grade or equivalent)	MLS
100% ethanol (for Wash Solution Concentrate; ACS reagent grade or equivalent)	MLS

General guidelines

IMPORTANT! Instrument should be decontaminated before use.

- For the KingFisher™ Duo Prime or KingFisher™ Apex instruments, use the ultraviolet treatment option on the instrument for at least one hour before using the instrument for the procedure.
- For the KingFisher™ Flex instrument, wipe down the process chamber with RNase*Zap*™ RNase Decontamination Solution or alcohol before using the instrument for the procedure.

- Perform all steps at room temperature (20–25°C), unless otherwise noted.
- Precipitate can occur in the 2X Hybridization Buffers and binding buffers provided with this kit. If precipitate is observed, warm the 2X Hybridization Buffer at 50°C with occasional mixing until the solution is clear (5–10 minutes).
- (*Optional*): To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

Protocol for KingFisher™ Apex and KingFisher™ Flex

Set up the instrument

1. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	96 deep-well magnetic head
Heat block	96 well deep-well heat block

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields and potential harm to the instrument.

2. Ensure that the proper program is loaded onto the instrument.
   a. KingFisher™ Apex: Select the **RiboMinus_Bacteria_2.0_rRNA_Depletion_v1** program from the Protocol Cloud Library.

   b. KingFisher™ Flex: Download the **RiboMinus_Bacteria_2.0_Flex_v1** program from the product page.

Set up the processing plates

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
RiboMinus™ Magnetic Beads	2	Deep-well	Streptavidin beads	500 µL
Bead Wash	3	Deep-well	Nuclease free water	1,000 µL
Wash Solution Concentrate with 100% ethanol (see page 6)	4	Deep-well	RNA wash buffer	300 µL
Elution Plate	5	Deep-well	Nuclease free water	50 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a deep-well plate		

Note: Load all plates (including the sample plate) into the instrument immediately.

Prepare Sample Plate

1. Add 125 µL of 2X Hybridization Buffer to sample plate wells.
2. Add total RNA (100 ng to 5 µg) to sample plate wells.
3. Add the RiboMinus™ Pan-Prokaryote Probe Mix to sample plate wells.

Total RNA input	RiboMinus™ Pan-Prokaryote Probe Mix
<1 µg	1 µL
1–5 µg	3 µL

4. Add nuclease free water to bring up the total volume to 250 µL.
5. Load the sample plate in plate position 1.

Bind, wash, then elute ribosomal RNA depleted total RNA

1. Select the program **RiboMinus_Bacteria_2.0_rRNA_Depletion_v1** (for the KingFisher™ Apex), or **RiboMinus_Bacteria_2.0_Flex_v1** (for the KingFisher™ Flex) on the instrument.

2. Start the run, then load the prepared plates into position when prompted by the instrument.

3. When the instrument pauses, add 10 µL of Nucleic Acid Binding Beads (magnetic clean up beads), 240 µL of Binding Solution Concentrate and 250 µL of 100% isopropanol to the sample wells on the sample plate. Click **OK** on the instrument to proceed with clean up.

   IMPORTANT! This step must be performed with isopropanol for the automated protocol.

4. After the protocol is complete (~19 minutes after adding Binding Bead Mix), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to the final tube or plate of choice for final storage.

The ribosomal RNA depleted total RNA is ready for immediate use. Alternatively, store the plate at –80°C for long term storage.

Note: Check stored plates for discoloration before use. The eluate can contain tiny amounts of residual magnetic beads which can be separated by putting the elution plate on a magnetic stand and re-collecting the eluate.

Protocol for KingFisher™ Duo Prime

Set up the instrument

1. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	12-tip magnetic head
Heat block	12 well standard heat block

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields.

2. Ensure that the proper program (**RiboMinus_Bacteria_2.0_Duo_v1**) has been downloaded from the product page and loaded onto the instrument.

Prepare sample wells in Row A

1. Add 125 µL of 2X Hybridization Buffer to sample wells in Row A.
2. Add total RNA (100 ng to 5 µg) to sample wells.
3. Add the RiboMinus™ Pan-Prokaryote Probe Mix to sample wells.

Total RNA input	RiboMinus™ Pan-Prokaryote Probe Mix
<1 µg	1 µL
1–5 µg	3 µL

4. Add nuclease free water to bring up the total volume to 250 µL.

Set up the processing plates

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Row ID	Plate Row	Reagent	Volume per well
Samples for depletion	A	2X Hybridization Buffer Total RNA Pan-prokaryotic probe	250 µL
RiboMinus™ Magnetic Beads	B	Streptavidin beads	500 µL
Bead Wash	C	Nuclease free water	1,000 µL
Wash Solution Concentrate with 100% ethanol (see page 6)	D	RNA wash buffer	300 µL
Empty	E	—	—
Empty	F	—	—
Empty	G	—	—
Tip comb	H	Tip comb	—

IMPORTANT! Load the plate including the elution strip (containing 50 µL of elution volume per well) into the instrument immediately after the Sample Row has been prepared.

Bind, wash, then elute ribosomal RNA depleted total RNA

1. Select the program **RiboMinus_Bacteria_2.0_Duo_v1** on the instrument.
2. Start the run, then load the prepared plate and elution strip into position when prompted by the instrument.
3. When the instrument pauses, add 10 µL of Nucleic Acid Binding Beads (magnetic clean up beads), 240 µL of Binding Solution Concentrate and 250 µL of 100% isopropanol to the sample wells in Row A. Click **OK** on the instrument to proceed to Bind/wash and elute steps.

   IMPORTANT! This step must be performed with isopropanol for the automated protocol.

4. After the protocol is complete (~19 minutes after adding Binding Bead Mix), immediately remove the plate from the instrument and cover the plate or transfer the eluate to the final tube or plate of choice for final storage.

The ribosomal RNA depleted total RNA is ready for immediate use. Alternatively, store the plate at –80°C for long term storage.

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.

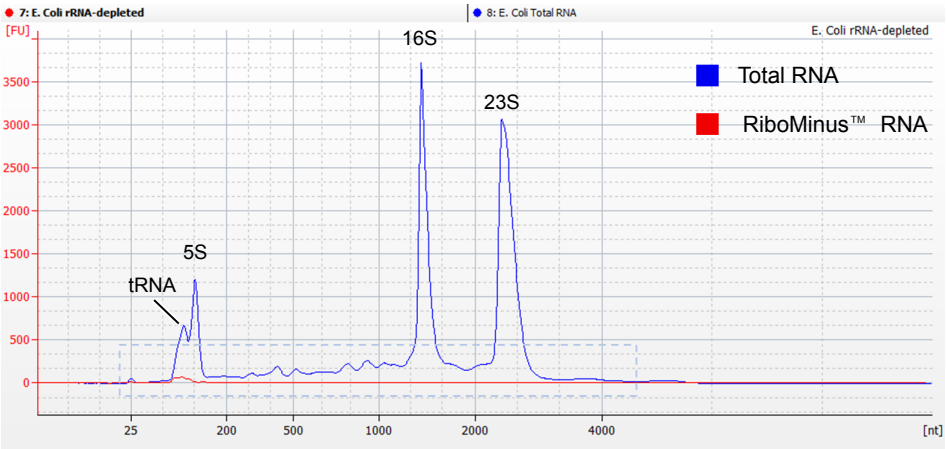

Figure 1 Agilent™ RNA 6000 Pico Assay shows an overlay of RiboMinus™ RNA with *E. coli* total RNA. The RiboMinus™ RNA sample was diluted 1:5 and 1 µL was ran along with 1 µL of a 1:5 dilution of *E. coli* total RNA Samples. 5 µg total RNA input into the depletion reaction.

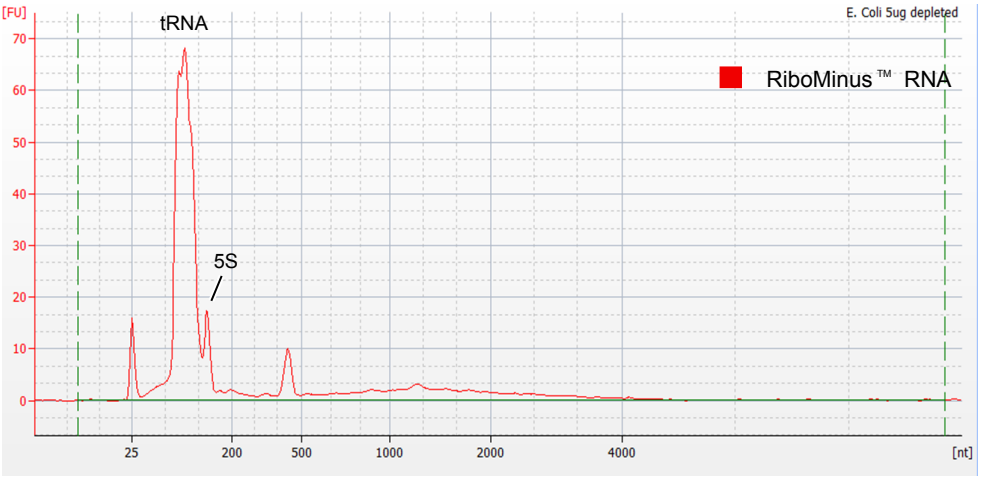

Figure 2 Agilent™ RNA 6000 Pico Assay detailed trace of RiboMinus™ RNA shows absence of rRNA peaks.

A 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.
Unusually higher than expected rRNA reads remaining	Contamination of rRNA carried over from previous total RNA isolations on instrument.	Decontaminate instrument before use. • For the KingFisher™ Duo Prime or KingFisher™ Apex instruments, use the ultraviolet treatment option on the instrument for at least one hour before using the instrument for the procedure. • For the KingFisher™ Flex instrument, wipe down the process chamber with RNase*Zap*™ RNase Decontamination Solution or alcohol before using the instrument for the procedure.
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.

B

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

C 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

Customer and technical support

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- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- 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**.