

life

**RiboMinus™ Human/Mouse
Transcriptome Isolation Kit,
RiboMinus™ Transcriptome
Isolation Kit (Human/Mouse), and
RiboMinus™ Concentration Module**

For efficient transcriptome enrichment by
depleting large ribosomal RNA

Catalog Numbers K1500-01, K1500-02, and K1500-05

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Experienced Users Procedure

RiboMinus™ Human/Mouse Transcriptome Isolation Kit

This quick reference protocol is included for experienced users of the RiboMinus™ Human/Mouse Transcriptome Isolation Kit (Cat. no. K1550-01). If you are a first time user, follow the detailed protocol in this manual.

Step	Action						
Isolating total RNA	Isolate high-quality total RNA from cells or tissues using a method of choice prior to starting the protocol. You will need 2–10 µg total RNA per reaction.						
Selective hybridization	<ol style="list-style-type: none"> Perform hybridization of your total RNA sample by adding the following reagents to a sterile, RNase-free 1.5 mL microcentrifuge tube: <table data-bbox="335 600 899 706"> <tr> <td>Total RNA (2–10 µg):</td> <td>20 µL</td> </tr> <tr> <td>RiboMinus™ Probe (100 pmol/µL):</td> <td>8 µL</td> </tr> <tr> <td>Hybridization Buffer (B5):</td> <td>300 µL</td> </tr> </table> Incubate the tube at 70–75°C for 5 minutes to denature the RNA. Cool the sample to 37°C over a period of 30 minutes by placing the tube in a 37°C water bath. During the sample cooling process, proceed to Preparing RiboMinus™ Magnetic Beads. 	Total RNA (2–10 µg):	20 µL	RiboMinus™ Probe (100 pmol/µL):	8 µL	Hybridization Buffer (B5):	300 µL
Total RNA (2–10 µg):	20 µL						
RiboMinus™ Probe (100 pmol/µL):	8 µL						
Hybridization Buffer (B5):	300 µL						
Preparing RiboMinus™ Magnetic Beads	<ol style="list-style-type: none"> Resuspend the RiboMinus™ Magnetic Beads in the bottle. Pipet 500 µL of the bead suspension into a sterile, RNase-free, 1.5 mL microcentrifuge tube. Place the tube with the bead suspension on a magnetic stand for 1 minute, then gently aspirate and discard the supernatant. Add 500 µL sterile, RNase-free water to the beads and resuspend the beads. Place the tube on a magnetic stand for 1 minute, then gently aspirate and discard the supernatant. Repeat step 4 once. Resuspend the beads in 500 µL of Hybridization Buffer (B5). Place the tube on a magnetic stand for 1 minute, then gently aspirate and discard the supernatant. Resuspend th beads in 200 µL Hybridization Buffer (B5) and keep the beads at 37°C until use. 						

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Experienced Users Procedure, Continued

Step	Action
Removing rRNA	<ol style="list-style-type: none"> 1. Transfer the cooled RNA sample (from Selective hybridization) to the tube of prepared RiboMinus™ Magnetic beads (from Preparing RiboMinus™ Magnetic Beads), and mix well. 2. Incubate the tube at 37°C for 15 minutes. During incubation, gently mix the contents occasionally. 3. Place the tube on a magnetic stand for 1 minute to pellet the rRNA-probe complex. The supernatant contains the RiboMinus™ RNA fraction. 4. Transfer the supernatant (~ 528 µL) to a tube with a capacity of 3X the volume of the supernatant. 5. Resuspend the beads in 50 µL of Hybridization Buffer (B5). Place the tube on a magnetic stand for 1 minute, then transfer the supernatant to the tube from step 4 (for a total supernatant volume of ~575 µL).
Concentrating RiboMinus™ RNA	<ol style="list-style-type: none"> 1. Add 500 µL Binding Buffer (L3) and 600 µL 96-100% ethanol to the RiboMinus™ RNA (from Removing rRNA). Mix well. 2. Bind ~700 µL sample from step 1 by applying it to the spin column. Centrifuge the column at 12,000 × g for 1 minute at room temperature. Discard the flow through. 3. Repeat the binding step twice to bind the remainder of the sample onto the column. 4. Wash the column with 600 µL of Wash Buffer (W5) with ethanol (see page 13). Centrifuge the column at 12,000 × g for 1 minute at room temperature. Discard the flow through. 5. Repeat the wash step once. 6. Discard the tube and place the column into a clean 2.0-mL Wash Tube (supplied with the kit). 7. Centrifuge the column at maximum speed for 2–3 minutes at room temperature to remove residual Wash Buffer (W5). Place the column in a 1.7-ml Recovery Tube. 8. Elute with 50–100 µL of Sterile, RNase-free water (pH >7.0). Incubate the column at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute. The Recovery Tube contains purified RiboMinus™ RNA. 9. Use the RiboMinus™ RNA for the desired downstream application, or store at –80°C.

Experienced Users Procedure, Continued

RiboMinus™ Transcriptome Isolation Kit (Human/Mouse)

This quick reference protocol is included for experienced users of the RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) (Cat. no. K1550-02). If you are a first time user, follow the detailed protocol in this manual.

Step	Action						
Isolating total RNA	Isolate high-quality total RNA from cells or tissues using a method of choice prior to starting the protocol. You will need 2–10 µg total RNA per reaction.						
Selective hybridization	<ol style="list-style-type: none"> 1. Perform hybridization of your total RNA sample by adding the following reagents to a sterile, RNase-free 1.5 mL microcentrifuge tube: <table data-bbox="338 581 899 683"> <tr> <td>Total RNA (2–10 µg):</td> <td><20 µL</td> </tr> <tr> <td>RiboMinus™ Probe (100 pmol/µL):</td> <td>8 µL</td> </tr> <tr> <td>Hybridization Buffer (B5):</td> <td>300 µL</td> </tr> </table> 2. Incubate the tube at 70–75°C for 5 minutes to denature the RNA. Cool the sample to 37°C over a period of 30 minutes by placing the tube in a 37°C water bath. 3. During the sample cooling process, proceed to Preparing RiboMinus™ Magnetic Beads. 	Total RNA (2–10 µg):	<20 µL	RiboMinus™ Probe (100 pmol/µL):	8 µL	Hybridization Buffer (B5):	300 µL
Total RNA (2–10 µg):	<20 µL						
RiboMinus™ Probe (100 pmol/µL):	8 µL						
Hybridization Buffer (B5):	300 µL						
Preparing RiboMinus™ Magnetic Beads	<ol style="list-style-type: none"> 1. Resuspend the RiboMinus™ Magnetic Beads by thorough vortexing of the bottle. 2. Pipet 500 µL of the bead suspension into a sterile, RNase-free, 1.5-mL microcentrifuge tube. 3. Place the tube with the bead suspension on a magnetic stand for 1 minute, then gently aspirate and discard the supernatant. 4. Add 500 µL sterile, RNase-Free Water to the beads and resuspend the beads. Place the tube on a magnetic stand for 1 minute, then gently aspirate and discard the supernatant. 5. Repeat step 4 once. 6. Resuspend the beads in 500 µL of Hybridization Buffer (B5). Place the tube on a magnetic stand for 1 minute, then gently aspirate and discard the supernatant. 7. Resuspend the beads in 200 µL of Hybridization Buffer (B5) and keep the beads at 37°C until use. 						

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Experienced Users Procedure, Continued

Step	Action
Removing rRNA	<ol style="list-style-type: none"> 1. Transfer the cooled RNA sample (from Selective hybridization) to the tube of prepared RiboMinus™ Magnetic beads (from Preparing RiboMinus™ Magnetic Beads), and mix well. 2. Incubate the tube at 37°C for 15 minutes. During incubation, gently mix the contents occasionally. 3. Place the tube on a magnetic stand for 1 minute to pellet the rRNA-probe complex. The supernatant contains the RiboMinus™ RNA fraction. 4. Transfer the supernatant (~ 528 µL) to a tube with a capacity of 3X the volume of the supernatant. 5. Proceed to Concentrating RiboMinus™ RNA using the RiboMinus™ Concentration Module, or see page 18–19 to concentrate the RiboMinus™ RNA by ethanol precipitation.
Concentrating RiboMinus™ RNA using the RiboMinus™ Concentration Module	<ol style="list-style-type: none"> 1. Add 1X sample volume of the Binding Buffer (L3) and 1X sample volume of 96–100% ethanol to the RiboMinus™ RNA (from Removing rRNA). Mix well. 2. Bind ~700 µL sample from step 1 by applying it to the spin column. Centrifuge the column at 12,000 × g for 1 minute at room temperature. Discard the flow through. 3. Repeat the binding step twice to bind the remainder of the sample onto the column. 4. Wash the column with 200 µL Wash Buffer (W5) with ethanol (see page 13). Centrifuge the column at ≥12,000 × g for 1 minute at room temperature. Discard the flow through. 5. Repeat the wash step once. 6. Discard the tube and place the column into a clean 2.0-mL Wash Tube (supplied with the kit). 7. 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 1.5-mL Recovery Tube. 8. Elute with 10–15 µL of RNase-Free Water. Incubate the column at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute. The Recovery Tube contains purified RiboMinus™ RNA. 9. Use the RiboMinus™ RNA for the desired downstream application, or store at –80°C.

Kit Contents and Storage

Types of kits

This manual is supplied with the following products.

Product	Catalog no.
RiboMinus™ Human/Mouse Transcriptome Isolation Kit	K1550-01
RiboMinus™ Transcriptome Isolation Kit (Human/Mouse)	K1550-02
RiboMinus™ Concentration Module	K1550-05

All components of the RiboMinus™ Human/Mouse Transcriptome Isolation Kit, the RiboMinus™ Transcriptome Isolation Kit (Human/Mouse), and the RiboMinus™ Concentration Module are shipped at room temperature.

Upon receipt, store components as follows:

Shipping and storage

The RiboMinus™ Human/Mouse Transcriptome Isolation Kit (Cat. no. K1550-01) consists of two modules.

Product	Storage
RiboMinus™ Human/Mouse Module	4°C*
RiboMinus™ Concentration Module	Room temperature

The RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) (Cat. no. K1550-02) consists of a single module.

Product	Storage
RiboMinus™ Transcriptome Isolation Kit	4°C*

The RiboMinus™ Concentration Module (Cat. no. K1550-05) consists of a single module.

Product	Storage
RiboMinus™ Concentration Module	Room temperature

* For long-term storage, store the RiboMinus™ Human/Mouse Probe at -20°C.

Continued on next page

Kit Contents and Storage, Continued

Kit components

The components for each module in the RiboMinus™ Human/Mouse Transcriptome Isolation Kit, RiboMinus™ Transcriptome Isolation Kit (Human/Mouse), and the RiboMinus™ Concentration Module are listed in this section.

RiboMinus™ Human/Mouse Transcriptome Isolation Kit contents

The RiboMinus™ Human/Mouse Transcriptome Isolation Kit (Cat. no. K1550-01) consists of two modules. The components included in each module are listed below:

RiboMinus™ Human/Mouse Module	
Component	Amount
RiboMinus™ Magnetic Beads (12 mg/mL) in Phosphate Buffered Saline (PBS), pH 7.4 containing 0.01% Tween 20 and 0.09% sodium azide	3.3 mL
RiboMinus™ Human/Mouse Probe in ultrapure water (100 pmol/μL)*	50 μL
Hybridization Buffer (B5)	6.2 mL

* For long-term storage, store the RiboMinus™ Human/Mouse Probe at -20°C.

RiboMinus™ Concentration Module	
Component	Amount
Binding Buffer (L3)	3.3 mL
Wash Buffer (W5)	1.5 mL
Sterile, RNase-Free Water	6.6 mL
Spin Columns with Collection Tubes	6
Wash Tubes (2.0 mL)	6
Recovery Tubes (1.7 mL)	6

Sufficient reagents are provided in the kit to perform 6 reactions.

Note: Some reagents are provided in excess.

Continued on next page

Kit Contents and Storage, Continued

RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) contents

The RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) (Cat. no. K1550-02) consists of one module.

RiboMinus™ Human/Mouse Module	
Component	Amount
RiboMinus™ Magnetic Beads (12 mg/mL) in Phosphate Buffered Saline (PBS), pH 7.4 containing 0.01% Tween 20 and 0.09% sodium azide	3.3 mL
RiboMinus™ Human/Mouse Probe in ultrapure water (100 pmol/μL)*	50 μL
Hybridization Buffer (B5)	6.2 mL
RNase-Free Water	6.6 mL

* For long-term storage, store the RiboMinus™ Human/Mouse Probe at -20°C.

Sufficient reagents are provided in the kit to perform 6 reactions.

Note: Some reagents are provided in excess.

RiboMinus™ Concentration Module contents

The RiboMinus™ Concentration Module (Cat. no. K1550-05) consists of one module.

RiboMinus™ Concentration Module	
Component	Amount
Binding Buffer (L3)	3.3 mL
Wash Buffer (W5)	1.5 mL
Sterile, RNase-Free Water	6.6 mL
Spin Columns with Collection Tubes	6
Wash Tubes (2.0 mL)	6
Recovery Tubes (1.5 mL)	6

Sufficient reagents are provided in the kit to perform 6 reactions.

Note: Some reagents are provided in excess.

Intended use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Introduction

About the Kit

Introduction

The transcriptome is defined as the complete collection of transcribed elements of the genome (Ruan, Le Ber et al. 2004) and contains mRNA transcripts and non-mRNA transcripts, and transcriptome analysis is important in the understanding of function and regulation of biological pathways.

The RiboMinus™ Human/Mouse Transcriptome Isolation Kit, and RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) utilize a novel and efficient method for transcriptome isolation from human and mouse samples that works through selective removal of large ribosomal RNA (rRNA) from total RNA.

Standard RNA purification methods are not ideal for efficient transcriptome isolation. Total RNA isolation results in co-purification of large rRNA molecules that account for ~90–95% of RNA species in the sample. These species can result in generation of undesired products in subsequent experimental procedures.

Subsequent processing of total RNA using polyA-selection and/or cap-binding approaches do not enrich for the complete transcriptome, as RNA species that are not polyadenylated, or do not have a 5'-cap structure are not captured.

The RiboMinus™ Human/Mouse Transcriptome Isolation Kit, and RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) enable analysis of the whole transcriptome, because the method is not dependent on the polyadenylation status or presence of a 5'-cap structure on the RNA. It produces an isolated transcriptome that is >95% depleted of large 18S and 28S rRNA, and enriched in all RNA transcripts of interest.

Continued on next page

About the Kit, Continued

System overview

The RiboMinus™ Human/Mouse Transcriptome Isolation Kit, and RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) are used to selectively remove large 18S and 28S human and mouse rRNA molecules from total RNA. The resulting RNA fraction that is depleted of rRNA is termed RiboMinus™ RNA (see page 3 for details).

Starting with 10 µg of total RNA, the sample is hybridized with the biotin labeled RiboMinus™ Human/Mouse Probe containing 5–7 LNA® (Locked Nucleic Acid) monomers incorporated at specific locations (see page 26 for details). The rRNA/biotin labeled probe complex is then removed from the sample with streptavidin coated RiboMinus™ Magnetic Beads (see page 7 for details).

After rRNA removal, the RiboMinus™ RNA sample is concentrated by spin column-based centrifugation (page 11), or ethanol precipitation (page 16).

Spin column based concentration is performed using the RiboMinus™ Concentration Module. Binding conditions for the spin column are optimized for the RiboMinus™ RNA sample with ethanol and Binding Buffer (L3). The sample is loaded onto the spin column, and the RiboMinus™ RNA binds to the silica-based membrane in the column. Impurities are removed by washing with Wash Buffer (W5), and the RNA is eluted in sterile RNase free water.

The resulting RiboMinus™ RNA has >95% of large 18S and 28S rRNA molecules removed, enabling analysis of the transcriptome without interference from the rRNA.

Continued on next page

About the Kit, Continued

RiboMinus™ RNA

The rRNA depleted RNA fraction produced after processing the sample with the RiboMinus™ Human/Mouse Transcriptome Isolation Kit, or RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) is termed the RiboMinus™ RNA fraction.

The RiboMinus™ RNA fraction contains polyadenylated (polyA) mRNA, non-polyadenylated RNA, pre-processed RNA, tRNA, small rRNAs (5S rRNA, 5.8S rRNA), and may also contain regulatory RNA molecules such as microRNA (miRNA) and short interfering RNA (siRNA), snRNA, and other RNA transcripts of yet unknown function.

The RiboMinus™ RNA molecules are part of the transcriptome and are important in protein coding, signaling, structural support of subcellular elements, and transcriptional/post transcriptional regulation.

Advantages

Using the RiboMinus™ Human/Mouse Transcriptome Isolation Kit, and RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) to isolate RiboMinus™ RNA (rRNA depleted RNA) provides the following advantages:

- Rapid and efficient isolation of high-quality RiboMinus™ RNA using probes specific to 18S and 28S human and mouse rRNA
 - Specifically designed to isolate RiboMinus™ RNA enriched in polyadenylated (polyA) mRNA, non-polyadenylated RNA, pre-processed RNA, tRNA, and small rRNAs (5S rRNA, 5.8S rRNA)
 - Minimal contamination from rRNA molecules
 - Reliable performance of the RiboMinus™ RNA in downstream applications such as microarray analysis, cDNA library construction, and qRT-PCR
-

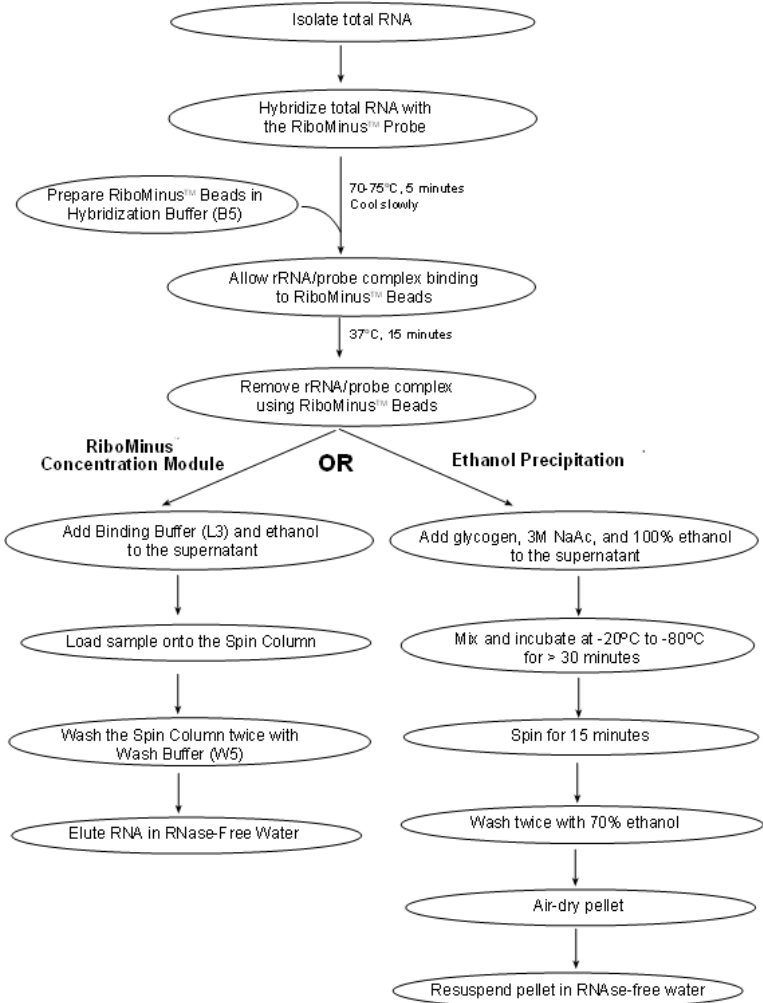
Downstream applications

The isolated RiboMinus™ RNA is suitable for use in downstream applications such as microarray analysis, qRT-PCR, and cDNA library construction

Experimental Overview

Introduction

The flow chart for isolating transcriptome using the RiboMinus™ Human/Mouse Transcriptome Isolation Kit, and the RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) is shown below.



Preparing Total RNA

Introduction

You will need to isolate high-quality total RNA from cells or tissues using a method of choice prior to using this kit. To obtain high-quality total RNA, follow the guidelines recommended below.

General handling of RNA

Observe the following guidelines to prevent RNase contamination:

- Use disposable, individually wrapped, sterile plasticware
 - Use only new, sterile pipette tips and microcentrifuge tubes
 - Wear protective gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin
 - Always use proper microbiological aseptic techniques when working with RNA
 - Use RNase AWAY® Reagent (page 25) to remove RNase contamination from surfaces
-

Total RNA isolation

Total RNA can be isolated from tissue or cells using the method of choice. We recommend isolating total RNA using the Micro-to-Midi™ Total RNA Purification System or TRIzol® Reagent available from Invitrogen (see page 25 for ordering information).

You will use 2–10 µg total RNA in >20 µL for each reaction. Resuspend isolated total RNA in DEPC-treated water accordingly (≥0.5 µg/µL).

Check the quality of your total RNA, including DNA contamination (see below). Store your total RNA at –80°C and avoid repeated freezing and thawing of total RNA.



If your downstream application requires DNA-free RNA, perform DNase-treatment of the total RNA **before** purifying RiboMinus™ RNA.

Continued on next page

Preparing Total RNA, Continued

Checking the total RNA quality

To check total RNA integrity, analyze ~0.5 µg of your RNA by agarose/ethidium bromide gel electrophoresis. You should see the following on an agarose gel:

- 28S rRNA band (5.0 kb for human; 4.7 kb for mouse) and 18S rRNA band (1.9 kb)
 - 28S band should be approximately twice the intensity of the 18S band
-

Methods

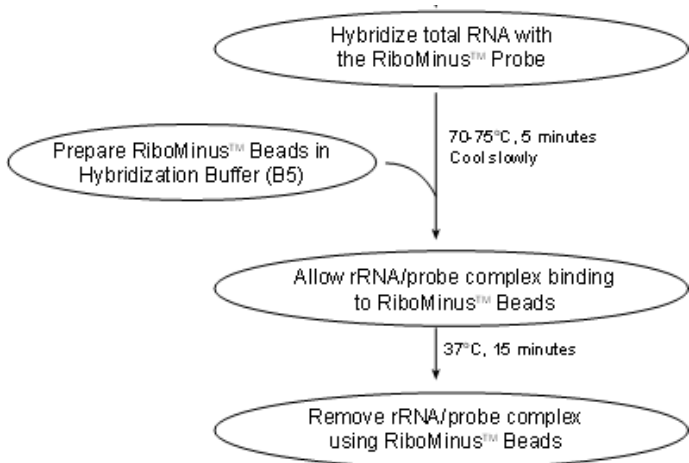
Selective Hybridization and Removal of rRNA

Introduction

Instructions are provided in this section for selective hybridization of rRNA to the RiboMinus™ Probe (see page 25 for details on the probe), and removal of rRNA using RiboMinus™ Magnetic Beads (see page 27 for details on the probe).

Experimental outline

The figure below depicts the experimental outline for hybridization of rRNA to specific probes and removal of rRNA.



Continued on next page

Selective Hybridization and Removal of rRNA, Continued

Materials needed

You will need the following items:

- Total RNA (see pages 5–6)
- Magna-Sep™ Magnetic Particle Separator (page 25) or equivalent
- RNase-free microcentrifuge tubes
- Water baths or heat blocks set to 70–75°C and 37°C
- Ice

Components supplied with the kit

- RiboMinus™ Magnetic Beads, keep on ice until use
 - RiboMinus™ Human/Mouse Probe, keep on ice until use
 - Hybridization Buffer (B5)
 - RNase-Free Water
-



Follow the recommendations for handling the RiboMinus™ Magnetic Beads below for best results:

- During the mixing and washing steps of the magnetic beads, mix beads by using a vortex. A low speed centrifuge pulse may be required to remove beads stuck in the tube cap. **Avoid mixing by pipetting up and down as it results in bead loss.**
 - During all washing steps with beads, add water or buffer to the tube containing beads while the tube is still on a magnetic stand to prevent drying of beads. Remove the tube from the magnet and resuspend the beads as described above. Do not allow the beads to dry as drying reduces the bead efficiency.
 - To aspirate the supernatant after bead washing, place the pipette tip at the opposite side of the tube, away from the beads. Carefully remove the supernatant without disturbing or removing any beads.
 - Do not submerge the magnetic stand in water. To clean the magnetic stand, spray the stand with ethanol and wipe it with a paper towel.
-

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Selective Hybridization and Removal of rRNA, Continued

Hybridization step

Instructions are provided below to perform hybridization for 2–10 µg of your total RNA sample with the RiboMinus™ Human/Mouse Probe.

To process >10 µg total RNA sample, it is recommended to divide the RNA into two or more samples, each containing <10 µg of total RNA.

1. Set a water bath or heat block to 70–75°C.
2. Add the following reagents to a sterile, RNase-free 1.5-mL microcentrifuge tube,;

Total RNA (2–10 µg):	≤20 µL
RiboMinus™ Probe (100 pmol/µL):	8 µL
Hybridization Buffer (B5):	300 µL
3. Incubate the tube at 70–75°C for 5 minutes to denature the RNA.
4. Allow the sample to cool to 37°C slowly over a period of 30 minutes by placing the tube in a 37°C water bath.
Note: Slow cooling is important to promote sequence-specific hybridization. **Do not** cool the sample quickly by placing the tube in cold water.
5. While the sample is cooling down, prepare the magnetic beads (page 10).

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Selective Hybridization and Removal of rRNA, Continued

Preparing RiboMinus™ Magnetic Beads

Follow the recommendations on page 8 for handling beads and performing the washing steps. Prepare the RiboMinus™ Magnetic Beads as follows:

1. Resuspend the RiboMinus™ Magnetic Beads in the bottle by thoroughly vortexing.
 2. Pipet 500 µL of the bead suspension into a sterile, RNase-free, 1.5-mL microcentrifuge tube.
 3. Place the tube with the bead suspension on a magnetic separator for 1 minute. The beads will settle against the side of the tube that faces the magnet. Aspirate and discard the supernatant.
 4. Add 500 µL sterile, RNase-Free Water supplied with the kit to the beads and resuspend the beads by vortexing.
 5. Place the tube on a magnetic separator for 1 minute. Gently aspirate and discard the supernatant.
 6. Repeat steps 4–5 once.
 7. Resuspend beads in 500 µL Hybridization Buffer (B5). Place the tube on a magnetic separator for 1 minute. Gently aspirate and discard the supernatant.
 8. Resuspend beads in 200 µL Hybridization Buffer (B5) and keep the beads at 37°C until use.
-

Selective Hybridization and Removal of rRNA, Continued

Removing rRNA

1. Set a water bath or heat block to 37°C.
 2. After the hybridized sample (from step 4, page 9) has cooled to 37°C, briefly centrifuge the tube to collect the sample to the bottom of the tube.
 3. Transfer the sample (~328 µL) to the washed and resuspended RiboMinus™ Magnetic beads from step 8, above. Mix well by vortexing the tube repeatedly.
 4. Incubate the tube at 37°C for 15 minutes. During incubation, gently mix the contents occasionally. Briefly centrifuge the tube to collect the sample to the bottom of the tube.
 5. Place the tube on a magnetic separator for 1 minute to pellet the rRNA-probe complex. **Do not discard the supernatant.** The supernatant contains RiboMinus™ RNA.
 6. Transfer the supernatant (~ 528 µL) to a tube capable of holding 3X the volume of the supernatant.
 7. **RiboMinus™ Human/Mouse Transcriptome Isolation Kit (Cat. no. K1550-01) only:** Resuspend the beads in 50 µL of Hybridization Buffer (B5). Place the tube on a magnetic stand for 1 minute, then transfer the supernatant to the tube from step 6 (total supernatant volume of ~575 µL).
-

Concentrating the RiboMinus™ RNA

Concentrate the purified RiboMinus™ RNA using the RiboMinus™ Concentration Module.

- See pages 14–15 for the concentration protocol for the **RiboMinus™ Human/Mouse Transcriptome Isolation Kit (Cat. no. K1550-01)**.
 - See pages 16–17 for the concentration protocol for the **RiboMinus™ Human/Mouse Transcriptome Isolation Kit (Cat. no. K1550-01)**.
 - or by ethanol precipitation (pages 18–19).
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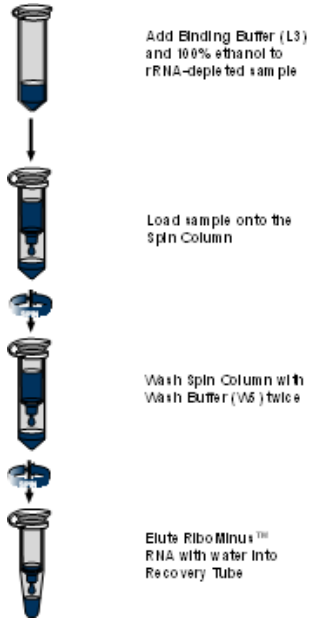
Using RiboMinus™ Concentration Module to Concentrate RiboMinus™ RNA

Introduction

The RiboMinus™ Concentration Module is designed to concentrate RiboMinus™ RNA purified using the RiboMinus™ Human/Mouse Transcriptome Isolation Kit, or the RiboMinus™ Transcriptome Isolation Kit (Human/Mouse). Concentration is performed using a spin column-based centrifugation protocol, and can be completed in a total time of **10–15 minutes**.

Experimental outline

The following figure depicts the experimental outline for concentrating the RiboMinus™ RNA using a spin-column based centrifugation procedure.



Using RiboMinus™ Concentration Module to Concentrate RiboMinus™ RNA, Continued

Materials needed

Materials required, but not supplied with the RiboMinus™ Concentration Module:

- RiboMinus™ RNA sample
 - Step 7, page 11 for the RiboMinus™ Human/Mouse Transcriptome Isolation Kit (Cat. no. K1550-01), OR
 - Step 6, page 11 for the RiboMinus™ Transcriptome Isolation Kit (Human/ Mouse) (Cat. no. K1550-02).
- 96–100% ethanol
- Microcentrifuge capable of centrifuging $>12,000 \times g$

Components supplied with the RiboMinus™ Concentration Module:

- Binding Buffer (L3)
 - Wash Buffer (W5)
 - RNase-Free Water
 - Spin Column with Collection Tubes
 - Wash Tubes
 - Recovery Tubes
-

Caution: The RiboMinus™ Concentration Module Binding Buffer (L3) contains 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.

Preparing Wash Buffer (W5)

Add 6 mL 96–100% ethanol to 1.5 mL Wash Buffer (W5) included with the kit. Store the Wash Buffer (W5) with ethanol at room temperature.

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Using RiboMinus™ Concentration Module to Concentrate RiboMinus™ RNA, Continued

RiboMinus™ Human/Mouse Transcriptome Isolation Kit

Use the following protocol for concentrating RiboMinus™ RNA purified using the RiboMinus™ Human/Mouse Transcriptome Isolation Kit (Cat. no. K1550-01).

If you are using RiboMinus™ RNA purified using the RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) (Cat. no. K1550-02), see page 16.

Binding Step

1. Add 500 µL of Binding Buffer (L3) and 600 µL 96-100% ethanol to the sample from Step 7, page 11, for a total volume of ~1.6 mL. Mix well by vortexing.
 2. Remove a Spin Cartridge in a Collection Tube from the package.
 3. Load 700 µL sample containing Binding Buffer (L3) and ethanol to the cartridge.
 4. Centrifuge the cartridge at $12,000 \times g$ for 1 minute at room temperature. Discard the flow through.
 5. Repeat the steps 3–4 twice to load the remaining sample from step 1 onto the column.
 6. Proceed to **Washing Step**.
-

Washing step

1. Add 600 µL Wash Buffer (W5) with ethanol (page 13) to the cartridge.
 2. Centrifuge the cartridge at $12,000 \times g$ for 1 minute at room temperature. Discard the flow through.
 3. **Repeat** the wash step with 600 µL Wash Buffer (W5) with ethanol.
 4. Discard the collection tube and place the cartridge into a clean 2.0-mL Wash Tube (supplied with the kit).
 5. Centrifuge the cartridge at maximum speed for 2–3 minutes at room temperature to remove any residual Wash Buffer (W5). Discard the Wash Tube.
 6. Proceed to **Elution Step**.
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Using RiboMinus™ Concentration Module to Concentrate RiboMinus™ RNA, Continued

Elution Step

1. Place the Spin Cartridge in a clean 1.7-mL Recovery Tube (supplied with the kit).
2. Add 50–100 µL of Sterile, RNase-free water (pH >7.0) to the center of the column. Incubate the cartridge at room temperature for 1 minute.
3. Centrifuge the cartridge at maximum speed for 1 minute at room temperature.

Note: If you performed elution with 50 µL water, you can perform a second elution with another 50 µL of Sterile, RNase-free water (pH >7.0), if desired.

The Recovery tube contains purified RiboMinus™ RNA depleted of rRNA. Remove and discard the cartridge.

4. Place the RiboMinus™ RNA on ice to proceed to desired downstream application, or store the RiboMinus™ RNA at –80°C for later use.

See pages 20–22 to analyze yield and quality of the RiboMinus™ RNA.

Using RiboMinus™ Concentration Module to Concentrate RiboMinus™ RNA, Continued

RiboMinus™ Transcriptome Isolation Kit (Human/Mouse)

Use the following protocol for concentrating RiboMinus™ RNA purified using the RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) (Cat. no. K1550-02).

If you are using RiboMinus™ RNA purified using the RiboMinus™ Human/Mouse Transcriptome Isolation Kit (Cat. no. K1550-01), see page 14.

Binding step

1. To the sample from step 6, page 11, add 1X sample volume of the Binding Buffer (L3) and 1X sample volume of 96–100% ethanol. Mix well by vortexing.
 2. Load 700 µL of the sample containing Binding Buffer (L3) and ethanol to the column.
 3. Centrifuge the column at $\geq 12,000 \times g$ for 1 minute at room temperature. Discard the flow through.
 4. Repeat the steps 2–3 twice to load the remaining sample from step 1 onto the column.
 5. Proceed to **Washing Step**.
-

Washing step

1. Add 200 µL Wash Buffer (W5) with ethanol (see page 13) to the column.
 2. Centrifuge the column at $\geq 12,000 \times g$ for 1 minute at room temperature. Discard the flow through.
 3. Repeat the wash step with 200 µL Wash Buffer (W5) with ethanol.
 4. Discard the collection tube and place the column into a clean 2.0-mL Wash Tube (supplied with the kit).
 5. Centrifuge the column at maximum speed for 2–3 minutes at room temperature to remove any residual Wash Buffer (W5). Discard the Wash Tube.
 6. Proceed to **Elution Step**.
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Using RiboMinus™ Concentration Module to Concentrate RiboMinus™ RNA, Continued

Elution step

1. Place the Spin Column in a clean 1.5-mL Recovery Tube supplied with the kit.
2. Add 10–15 μL of RNase-Free Water to the center of the column. Incubate the column at room temperature for 1 minute.
3. Centrifuge the column at maximum speed for 1 minute at room temperature.

The Recovery tube contains purified RiboMinus™ RNA depleted of rRNA. Remove and discard the cartridge.

4. Place the RiboMinus™ RNA on ice to proceed to desired downstream application, or store the RiboMinus™ RNA at -80°C for later use.

See pages 20–22 to analyze yield and quality of the RiboMinus™ RNA.

Using Ethanol Precipitation to Concentrate RiboMinus™ RNA

Introduction

This section includes a protocol for **Ethanol Precipitation** to concentrate RiboMinus™ RNA purified with the RiboMinus™ Transcriptome Isolation Kit (Human/Mouse). At least 1 hour is required to perform the ethanol precipitation.

Materials needed

- RiboMinus™ RNA sample (step 7, page 11)
 - Glycogen, 20 µg/µL (see page 25)
 - 3 M sodium acetate in RNase-free water
 - 96–100% cold ethanol
 - 70% cold ethanol
 - RNase-free water
 - Sterile, RNase-free microcentrifuge tubes
 - Microcentrifuge capable of centrifuging >12,000 × g
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Using Ethanol Precipitation to Concentrate RiboMinus™ RNA, Continued

Ethanol precipitation

1. Transfer the RiboMinus™ RNA sample into a clean RNase-free 2-mL microcentrifuge tube.
 2. Add the following reagents to the RNA sample:

Glycogen (20 µg/µL)	1 µL
3 M sodium acetate	0.1X sample volume
100% ethanol	2.5X sample volume
 3. Mix well and incubate at –20 or –80°C for a minimum of 30 minutes.
 4. Centrifuge the tube for 15 minutes $\geq 12,000 \times g$ at 4°C.
 5. Carefully discard the supernatant without disturbing the pellet.
 6. Add 500 µL 70% cold ethanol.
 7. Centrifuge the tube for 5 minutes $\geq 12,000 \times g$ at 4°C.
 8. Carefully discard the supernatant without disturbing the pellet.
 9. Repeat steps 6–8 once.
 10. Air-dry the pellet for ~5 minutes (do not completely dry the pellet).
 11. Resuspend the RNA pellet in ~10–30 µL RNase-free water.
 12. Place the RiboMinus™ RNA on ice to proceed to desired downstream application, or store the RiboMinus™ RNA at –80°C for later use.

See pages 20–22 to analyze yield and quality of the RiboMinus™ RNA.
-

Analyzing RiboMinus™ RNA

RNA yield

The quantity of the purified RiboMinus™ RNA is easily quantitated using UV absorbance at 260 nm or Quant-iT™ RNA Assay Kit.

UV Absorbance

1. Dilute a small amount of the sample in 10 mM Tris-HCl, pH 7.0. Mix well. Transfer to a cuvette (1-cm path length).
Note: The RNA must be in a neutral pH buffer to accurately measure the UV absorbance.
2. Determine the OD₂₆₀ of the solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.0.

Calculate the amount of total RNA using the following formula:

$$\text{Total RNA } (\mu\text{g}) = \text{OD}_{260} \times 40 \mu\text{g} / (1 \text{ OD}_{260} \times 1 \text{ mL}) \times \text{dilution factor} \times \text{total sample volume (mL)}$$

The typical yield of RNA using the RiboMinus™ Human/Mouse Transcriptome Isolation Kit, and RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) is ~1 µg RNA from a 10 µg total RNA sample.

Quant-iT™ RNA Assay Kits

The Quant-iT™ RNA Assay Kit (see page 25) provides a rapid, sensitive, and specific method for RNA quantitation with minimal interference from DNA, protein, or other common contaminants that affect UV absorbance readings.

The kit contains a state-of-the-art quantitation reagent and pre-diluted standards for standard curve. The assay is performed in a microtiter plate format and is designed for reading in standard fluorescent microplate readers.

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Analyzing RiboMinus™ RNA, Continued

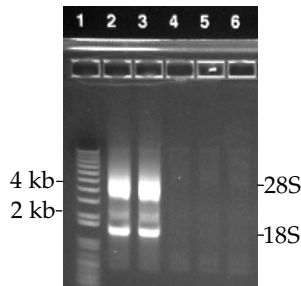
RNA quality

The RNA isolated using the RiboMinus™ Human/Mouse Transcriptome Isolation Kit, and RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) is of high-quality and is >95% depleted in rRNA species.

Depletion of rRNA can be verified by performing agarose gel electrophoresis of the sample (see below) or by using an Agilent Bioanalyzer™ (see page 22).

Gel electrophoresis to assess RNA quality

Agarose gel electrophoresis analysis shows depletion of 18S and 28S rRNA bands as compared to a control sample (see below for an example). Absence of contaminating DNA and RNA degradation may also be confirmed by agarose gel electrophoresis.



RiboMinus™ RNA was purified using 10 µg total RNA from 293F cells as described in this manual. Samples (5% of total eluate) were analyzed on a 0.8% E-Gel® agarose gel and imaged to visualize RNA.

- Lane 1: 1 µL 1 Kb Plus DNA Ladder
- Lane 2: Control sample 1 (purification procedure performed without using RiboMinus™ Magnetic Beads, or RiboMinus™ Human/Mouse Probe)
- Lane 3: Control sample 2 (purification procedure performed without using RiboMinus™ Human/Mouse Probe)
- Lanes 4–6: Purified RiboMinus™ RNA samples

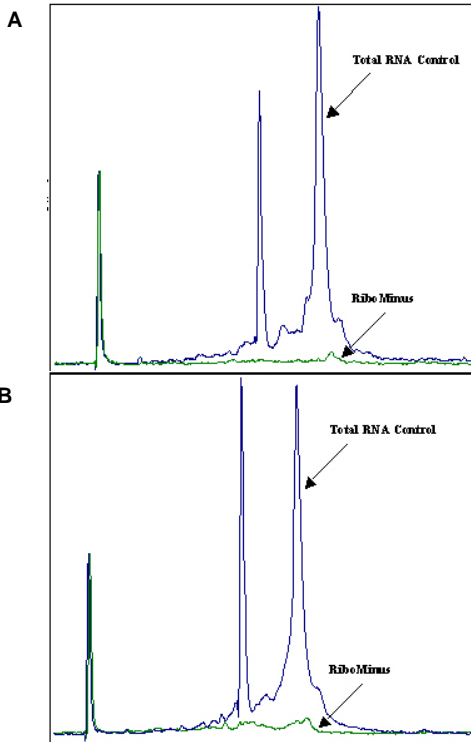
Lanes 4–6 show efficient removal of 18S and 28S rRNA bands from the purified RiboMinus™ RNA samples and Lane 3 shows absence of any non-specific rRNA removal.

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Analyzing RiboMinus™ RNA, Continued

Bioanalyzer™ to assess RiboMinus™ RNA quality

The efficiency of rRNA depletion in RiboMinus™ RNA, RNA degradation, and RNA concentration can be analyzed using an Agilent Bioanalyzer™ such as the Agilent 2100 Bioanalyzer™ with an RNA LabChip® separation system. In the example below, the Bioanalyzer™ data was used to show efficient removal of 18S and 28S rRNA from total RNA.



RiboMinus™ RNA was purified from 10 µg total RNA from 293F cells (panel A) or from mouse liver (panel B) using RiboMinus™ Transcriptome Isolation Kit (Human/Mouse) as described in this manual. The total RNA control samples were obtained by omitting the RiboMinus™ Magnetic Beads in the reaction mixture. Aliquots of 2% of the final elution volume of RiboMinus™ RNA and control total RNA were subjected to the bioanalysis using Agilent® 2100 Bioanalyzer™. The graph shows the removal of 18S and 28S rRNA from the purified RiboMinus™ RNA samples as compared to control samples.

Troubleshooting

Introduction

Review the table below to troubleshoot problems that you may encounter using the RiboMinus™ Human/Mouse Transcriptome Isolation Kit, or RiboMinus™ Transcriptome Isolation Kit (Human/Mouse).

Observation	Cause	Solution
Low RNA yield	Low RNA content	Various tissues have different RNA content and the yield is dependent on the sample.
	Incorrect binding conditions when using the RiboMinus™ Concentration Module	For efficient binding of RiboMinus™ RNA to the spin column, always add 1X sample volume of the Binding Buffer (L3) and 1X sample volume of 100% ethanol to the sample prior to loading onto the Spin Column.
	Ethanol not added to Wash Buffer (W5)	Be sure to add 96–100% ethanol to Wash Buffer (W5) as described on page 13.
	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 (page 20) to accurately measure the UV absorbance.
	Loss of pellet during to ethanol precipitation	<ul style="list-style-type: none">• Remove supernatant from RNA pellet carefully.• Use RiboMinus™ Concentration Module to concentrate the RiboMinus™ RNA.
Incomplete removal of rRNA	Too much total RNA used	The protocols are designed to purify RiboMinus™ RNA from 2–10 µg of total RNA. When using more than 10 µg of total RNA, divide the sample to two fractions, each containing <10 µg of total RNA.
	Low amount of magnetic beads or probe used	Be sure to use the recommended amounts of RiboMinus™ Probe and RiboMinus™ Magnetic Beads for efficient removal of rRNA.

Continued on next page

Troubleshooting, Continued

Observation	Cause	Solution
Incomplete removal of rRNA	Improper handling or drying of beads	To obtain the best results with RiboMinus™ Magnetic Beads, follow the guidelines on page 8 for washing and mixing the beads, and aspirating the supernatant. Do not allow the beads to dry as drying reduces the bead efficiency.
RNA degraded	RNA contaminated with RNase	Follow the guidelines on page 5 to prevent RNase contamination.
	Poor quality starting materials	Always use fresh samples or samples frozen at –80°C for isolation of total RNA. Be sure to check the quality of your total RNA prior to use.
Genomic DNA contamination	Total RNA contained genomic DNA	Perform DNase I digestion with the total RNA sample to remove any genomic DNA contamination before performing RiboMinus™ RNA purification.
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified RNA sample	<ul style="list-style-type: none"> • Traces of ethanol from the RiboMinus™ Concentration Module Wash Buffer (W5) or from ethanol precipitation can inhibit downstream enzymatic reactions. • To remove Wash Buffer (W5), discard Wash Buffer flow through from the collection tube. Reinsert the spin column into the collection tube and centrifuge the spin column at maximum speed for 2–3 minutes to completely dry the column. • If performing ethanol precipitation, make sure that ethanol is evaporated before resuspending the RiboMinus™ RNA pellet in RNase-free water.

Appendix

Product Specifications

System specifications

Starting Material:	2–10 µg total RNA in <20 µL
rRNA Removal:	>95%
RiboMinus™ RNA Yield:	~1 µg from 10 µg total RNA

RiboMinus™ Human/Mouse Probe

The RiboMinus™ Human/Mouse Probe is an oligonucleotide probe mixture containing 2 probes each specific for 18S rRNA and 28S rRNA (see page 25 for specifications). The probe is designed to hybridize with highly conserved regions of the human 18S and 28S rRNA. The probe also contains sufficient homology to hybridize efficiently against mouse rRNA.

Each probe is single-stranded and contains 5–7 LNA® (Locked Nucleic Acid) monomers incorporated at specific locations. The incorporation of LNA® (see next page for details on LNA®) into the oligonucleotide probe increases the depletion efficiency of the rRNA from the samples without increasing the amount of beads or probe concentration.

The 5'-end of each probe is conjugated to biotin to allow removal of rRNA/probe complexes by binding to streptavidin RiboMinus™ Magnetic Beads (see next page).

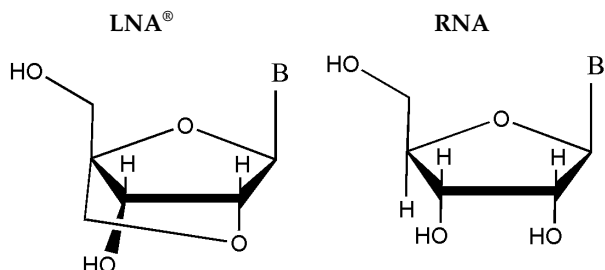
RiboMinus™ Probe specifications

Probe Contents:	2 probes each for 18S and 28S rRNA
Probe Specificity:	Human and mouse
Probe Size:	18–19 oligonucleotides
Probe Label:	5'-biotin label
LNA® Content:	Each probe contains 5–7 LNA® monomers in the oligonucleotide
Probe Mixture Concentration:	100 pmol/µL

Product Specifications, Continued

LNA[®] (Locked Nucleic Acid)

The structure of the LNA[®] (Locked Nucleic Acid) monomer (see figure below) consists of a ribonucleoside linked between the 2' oxygen and 4' carbon atom of the methylene ring (Braasch and Corey 2001).



This configuration locks the sugar backbone resulting in an increase in T_m (melting temperature).

Incorporation of 5–7 LNA[®] monomers into an oligonucleotide does not affect the ability of the oligonucleotide to bind DNA or RNA but increases the stability of the oligonucleotide/RNA complex (McTigue, Peterson et al. 2004). Oligonucleotides containing LNA[®] are used in hybridization assays requiring high specificity and reproducibility.

Product Specifications, Continued

RiboMinus™ Magnetic Beads

The RiboMinus™ Magnetic Beads are streptavidin-coated magnetic beads used for the removal of probe/rRNA complexes from the sample. The beads bind to the biotin-labeled probe complexed with rRNA or the probe alone.

The beads are 1 µm polystyrene beads with a magnetic core that is strong enough to separate the bound complex from the solvent in a short period of time (see page 25 for specifications). The beads do not promote non-specific binding of any other RNA molecules.

The size and the biotin binding capacity of the RiboMinus™ Magnetic Beads is optimized for use with this kit and results in >95% depletion of rRNA using 10 µg total RNA as the starting material. Avoid using any other streptavidin-coated magnetic beads with this kit.

RiboMinus™ Magnetic Bead specifications

The RiboMinus™ Magnetic Beads are streptavidin-coated magnetic beads.

Bead Binding Capacity:	>2500 pmoles free biotin per mg RiboMinus™ Magnetic Beads
Bead Size:	1 µm diameter
Magnet Particle:	Superparamagnetic polydisperse core-shell polystyrene particles
Concentration:	12 mg/mL
Specific Gravity:	1.1–1.4 g/cm ³

RiboMinus™ Concentration Module specifications

Binding Capacity:	~5 µg nucleic acid
Column Reservoir Capacity:	700 µL
Wash Tube Capacity:	2.0 mL
Recovery Tube Capacity:	1.7 mL or 1.5 mL
Centrifuge Compatibility:	Capable of centrifugation at >10,000 × g

Accessory Products

Additional products

The following products are also available from Invitrogen. For more details on these products, visit our Web site at www.invitrogen.com or contact Technical Support (page 29).

Product	Quantity	Catalog no.
RNase AWAY®	250 mL	10328-011
UltraPure™ DEPC-treated Water	1 L	750023
UltraPure™ DNase/RNase-Free Distilled Water	500 mL	10977-015
Quant-iT™ RNA Assay Kit	1000 assays	Q-33140
Micro-to-Midi™ Total RNA Purification System	50 reactions	12183-018
TRIzol® Reagent	100 mL	15596-026
DNase I	20,000 units	18047-019
DNase I, Amplification Grade	100 units	18068-015
Magna-Sep™ Magnetic Particle Separator	1	K1585-01
RiboMinus™ Yeast Transcriptome Isolation Kit (Yeast)	1	K1550-03
RiboMinus™ Transcriptome Isolation Kit (Bacteria)	1	K1550-04

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