## Analyzing RiboMinus™ RNA

The purified RiboMinus™ RNA is easily quantitated using UV absorbance at 260 nm or Quant-iT™ RNA Assay Kit. The RNA isolated using the RiboMinus™ Eukaryote Kit is of high-quality and is efficiently depleted in rRNA species. To verify the rRNA depletion, use a bioanalyzer 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 Agilent 2100 Bioanalyzer with the Agilent RNA 6000 Nano Kit. Agarose gel electrophoresis analysis can also show depletion of 18S and 28S rRNA bands as compared to a control sample. Absence of contaminating DNA and RNA degradation may also be confirmed by agarose gel electrophoresis. Analysis for low-input applications is optional. Where materials are limiting for low-input applications, it is recommended that all the RiboMinus™ RNA be used for your application.

## Troubleshooting

| Observation | Cause | Solution |
|---|---|---|
| Low RNA yield | Low RNA content | Follow the low-input protocol when using 100–500 ng of total RNA. |
| | Loss of pellet during ethanol precipitation | Remove supernatant from the RNA pellet carefully. |
| Incomplete removal of rRNA | Too much total RNA used | Divide the total RNA into two equally sized samples of <10 µg each when starting with more than 10 µg of total RNA. |
| | Insufficient amount of magnetic beads or probe used | Be sure to use the recommended amounts of RiboMinus™ Eukaryote Probe and RiboMinus™ Magnetic Beads for efficient removal of rRNA. |
| | Improper handling or drying of 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 the guidelines on page 1 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 isolating RiboMinus™ RNA. |
| Inhibition of downstream enzymatic reactions | Presence of ethanol in purified RNA sample | For ethanol precipitation, make sure that ethanol is evaporated before resuspending the RiboMinus™ RNA pellet in sterile, nuclease-free water. |

## Product Qualification and SDS

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/sds**.

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# RiboMinus™ Eukaryote Kit for RNA-Seq

**Catalog. no.** A10837-08 **Quantity** 8 prep **Store at 4ºC**

**Pub. Part no.** 100004590 MAN0000774 **Rev. Date** 2 December 2011

## Description

The RiboMinus™ Eukaryote Kit for RNA-Seq provides a novel and efficient method to isolate RNA molecules of the transcriptome devoid of large ribosomal RNA (rRNA) from total RNA for transcriptome analysis. The RiboMinus™ purification method is not dependent on the polyadenylation status or presence of a 5′-cap structure on the RNA which offer only a partial isolation of the transcriptome.

The ribosomal RNA depleted RNA fraction is termed the RiboMinus™ RNA fraction and is enriched in polyadenylated (polyA) mRNA, non-polyadenylated RNA, pre-processed RNA, tRNA, 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.

## Product Specifications

| | | | |
|---|---|---|---|
| **Starting material:** | • 1–10 µg total RNA (standard protocol)<br>• 100–500 ng total RNA (low-input protocol) | **Probe contents:** | 2 probes each for 5S, 5.8S, 18S, 28S rRNA |
| **rRNA removal:** | Up to 99% | **LNA® content:** | 3 LNA® monomers in each oligonucleotide probe |
| **RiboMinus™ RNA yield:** | • 1–2 µg from 10 µg human total RNA (standard protocol)<br>• ~100 ng from 500 ng human total RNA (low-input protocol) | **Bead binding capacity:** | ~4,000 pmoles free biotin per mg RiboMinus™ Magnetic Beads |
| **Probe specificity:** | Eukaryote* | **Bead size:** | 1 µm diameter |
| **Probe size:** | 22–25 oligonucleotides | **Bead concentration:** | ~10 mg/mL |
| **Probe label:** | 5′-biotin label | **Magnet particle:** | Superparamagnetic polydisperse core-shell polystyrene particles |

* The probe is designed to hybridize with highly conserved regions of 5S, 5.8S, 18S, and 28S rRNA from several eukaryotic species such as human, mouse, rat, drosophila, yeast, and others with zero mismatches. For a detailed list of species specificity for these probes, visit **www.lifetechnologies.com/rnapreps**

## Contents

| Components | A10837-02 | A10837-08 |
|---|---|---|
| RiboMinus™ Magnetic Beads (10 mg/mL) in phosphate buffered saline (PBS), pH 7.4 containing 0.01% Tween® 20 and 0.09% sodium azide | 2 mL | 6 mL |
| RiboMinus™ Eukaryote Probe in ultrapure water (15 pmol/µL) | 20 µL | 80 µL |
| Hybridization Buffer | 2.5 mL | 10 mL |
| DEPC-treated (RNase-Free) Water | 3 mL | 12 mL |

⚠ **CAUTION!** This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

## 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 AWAY® Reagent (Cat. no. 10328-011) to remove RNase contamination from surfaces.

**Product Use:** For research use only. Not intended for any animal or human therapeutic or diagnostic use.

## Materials Needed

| **Standard protocol** | **Low-input protocol** |
|---|---|
| Total RNA (1–10 µg) | Total RNA (100–500 ng) |
| DynaMag™ 2 Magnetic Stand (Cat. no. 123-21D) or equivalent | DynaMag™ 2 Magnetic Stand (Cat. no. 123-21D) or equivalent, and Magnetic Stand-96 (Cat. no. AM10027) |
| Water baths or heat blocks set to 70–75ºC and 37ºC | Water baths or heat blocks set to 70–75ºC and 37ºC |
| Glycogen, 20 µg/µL (Cat. no. 10814-010) | Agencourt RNAClean XP Kit (Beckman Cat. no. A639870) |
| 3 M sodium acetate in RNase-free water | MagMAX™ Lysis/Binding Solution Concentrate (Cat. no. AM8500) |
| 96–100% cold ethanol and 70% cold ethanol | 96–100% ethanol and 70% ethanol |
| Nuclease-free Water (Cat. no. AM9938) | Nuclease-free Water (Cat. no. AM9938) |
| (*Optional*) RiboMinus™ Concentration Module (Cat. no. K1550-05) | 1.2 mL Deep Well Plate, (96 round well) |
| | (*Optional*) RiboMinus™ Concentration Module (Cat. no. K1550-05) |

## General Guidelines

- During the mixing and washing steps with magnetic beads, mix beads by pipetting up and down or using a vortex set to low speed. A low speed centrifuge pulse may be required to remove beads stuck in the tube cap.
- 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.

## Prepare Total RNA

Each reaction requires 1–10 µg of high-quality total RNA in ≤10 µL of nuclease-free water (standard protocol), or 100–500 ng of RNA in 1–2 µL of nuclease-free water (low-input protocol). We recommend isolating total RNA using the PureLink™ RNA Mini Kit (Cat. no. 12183018A or 12183020) or TRIzol® Reagent (Cat. no. 15596-026). If your downstream application requires DNA-free RNA, perform DNase-treatment of the total RNA before purifying RiboMinus™ RNA. Check the quality of your total RNA, including DNA contamination.

## Hybridize Probe and Sample

Instructions are provided below to perform hybridization for 1–10 µg of your total RNA sample with the RiboMinus™ Eukaryote Probe. To process >10 µg total RNA sample, divide your sample into two samples, each containing <10 µg total RNA.

1. Set a water bath or heat block to 70–75ºC.
2. Add the following components to a sterile, RNase-free 1.5-mL microcentrifuge tube in the order listed:

| **Component** | **Standard protocol** | **Low-input protocol** |
|---|---|---|
| Hybridization Buffer | 100 µL | 30 µL |
| RiboMinus™ Probe (15 pmol/µL) | 10 µL | 1 µL |
| Total RNA | 1–10 µg ( <10 µL) | 100–500 ng (1–2 µL)* |

   * The low input RNA volume can be increased to 5 µL with a corresponding decrease in the amount of Hybridization Buffer to 27 µL.
3. Mix by gentle vortexing, and incubate the tube at 70–75ºC for 5 minutes to denature the RNA.
4. Immediately transfer the tubes to a 37ºC water bath/heat block, and allow the sample to cool to 37ºC over a period of 30 minutes. Slow cooling promotes sequence-specific hybridization. **Do not** cool samples quickly by placing the tubes in cold water.
5. While the sample is cooling, proceed to **Prepare Beads**.

## Prepare Beads

1. Resuspend RiboMinus™ Magnetic Beads in its bottle by thorough vortexing.
2. Pipet **750 µL** (**Standard**) or **75 µL** (**Low-input**) of bead suspension required for your sample 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 settle against the side of the tube facing the magnet. Gently aspirate and discard the supernatant.
4. Add **750 µL** (**Standard**) or **75 µL** (**Low-input**), sterile, nuclease-free water to the beads and resuspend beads by slow vortexing.
5. Place tube on a magnetic separator for 1 minute. Aspirate and discard the supernatant.
6. Repeat Steps 4–5 once.
7. Resuspend beads in **750 µL** (**Standard**) or **75 µL** (**Low-input**) Hybridization Buffer. Transfer **250 µL** (**Standard**) or **25 µL** (**Low-input**) of the beads to a new tube and maintain the tube at 37ºC for use at a later step.
8. Place the tube with **500 µL** (**Standard**) or **50 µL** (**Low-input**) of beads on a magnetic separator for 1 minute. Aspirate and discard the supernatant.
9. Resuspend beads in **200 µL** (**Standard**) or **20 µL** (**Low-input**) Hybridization Buffer and keep the beads at 37ºC for later use.



## Remove rRNA

1. After the RNA/ RiboMinus™ Probe mixture has cooled to 37ºC for 30 minutes, briefly centrifuge the tube to collect the sample to the bottom of the tube.
2. Transfer the sample (**~120 µL, Standard/~33 µL, Low-input**) to the prepared RiboMinus™ Magnetic beads from Step 9 (**Prepare Beads**). Mix well by pipetting up and down or low speed vortexing.
3. Incubate the tube at 37ºC for 15 minutes. During incubation, gently mix the contents occasionally.
4. 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. Place the tube with **250 µL** (**Standard**), or **25 µL** (**Low-input**) of beads from **Prepare Beads**, step 7 on a magnetic separator for 1 minute. Aspirate and discard the supernatant.
7. Add the supernatant containing RiboMinus™ RNA from step 5, above (**~320 µL, Standard/~53 µL, Low-input**) to the new tube of beads. Mix well by pipetting up and down or low speed vortexing.
8. Incubate the tube at 37ºC for 15 minutes. During incubation, gently mix the contents occasionally.
9. Briefly centrifuge the tube to collect the sample to the bottom of the tube.
10. 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**.
11. Transfer the supernatant (**~320 µL, Standard/~53 µL, Low-input**) containing RiboMinus™ RNA to a new tube.

## Concentrate RiboMinus™ RNA (Standard Protocol)

To ensure recovery of smaller (<200 nt) RNA, concentrate the RiboMinus™ RNA using ethanol precipitation, or the RiboMinus™ Concentration Module. Refer to the *Concentrating RiboMinus™ RNA Using the RiboMinus™ Concentration Module* insert for the protocol.

1. Transfer RiboMinus™ RNA sample to a clean, RNase-free 1.5-mL or 2-mL microcentrifuge tube.
2. Add the following components to the RiboMinus™ RNA:

   **Component**

   10 µL glycogen (20 µg/µL)

   0.1 volume (of eluted RNA sample) of 3 M sodium acetate

   2.5X volume of 100% ethanol
3. Mix well and incubate at –80ºC for ≥30 minutes.
4. Centrifuge the tube for 15 minutes at ≥12,000 × *g* at 4ºC. Carefully discard the supernatant without disturbing the pellet.
5. Add 500 µL of cold 70% ethanol.
6. Centrifuge the tube for 5 minutes at ≥12,000 × *g* at 4ºC. Carefully discard the supernatant without disturbing the pellet.
7. Repeat steps 5–6 once.
8. Air-dry the pellet for ~5 minutes. Resuspend the RiboMinus™ RNA pellet in 10–30 µL of nuclease-free water.

## Concentrate RiboMinus™ RNA (Low-Input Protocol)

Use Agencourt RNAClean XP beads to concentrate RiboMinus™ RNA for low-input depletion applications (recommended), or use the protocol from the *Concentrating RiboMinus™ RNA Using the RiboMinus™ Concentration Module* insert.

1. Resuspend Agencourt RNAClean XP beads by vortexing the bottle.
2. For each sample, add a 25 µL of the Agencourt RNAClean XP beads to one well of a 1.2 mL 96-well plate.
3. Place the plate on a magnetic stand for 2 minutes to pellet the beads, then carefully remove and discard the supernatant.
4. Add the following components to each well of the 96-well plate containing beads:

   **Component**

   ~53 µL RiboMinus™ RNA (entire volume from **Remove rRNA**, step 11)

   50 µL MagMAX™ Lysis/Binding Solution

   125 µL of 100% ethanol
5. Mix by pipetting, and incubate at room temperature for 5 minutes.
6. Place the place the plate on a magnetic stand for 2 minutes to pellet the beads, then carefully remove and discard the supernatant.
7. Keep the plate on the magnetic stand, and add 100 µL of **room temperature** 70% ethanol. Incubate for 30 seconds at room temperature and discard the supernatant.
8. Remove the plate from the magnetic stand and allow the samples to dry for 5 minutes.
9. Add 10 µL of nuclease-free water to the beads and mix by pipetting.
10. Incubate plate at room temperature for one minute, then place the plate on a magnetic stand for 2 minutes to pellet the beads.
11. Transfer the supernatant to a clean 1.5-mL tube. **Do not discard the supernatant. The supernatant contains RiboMinus™ RNA**.