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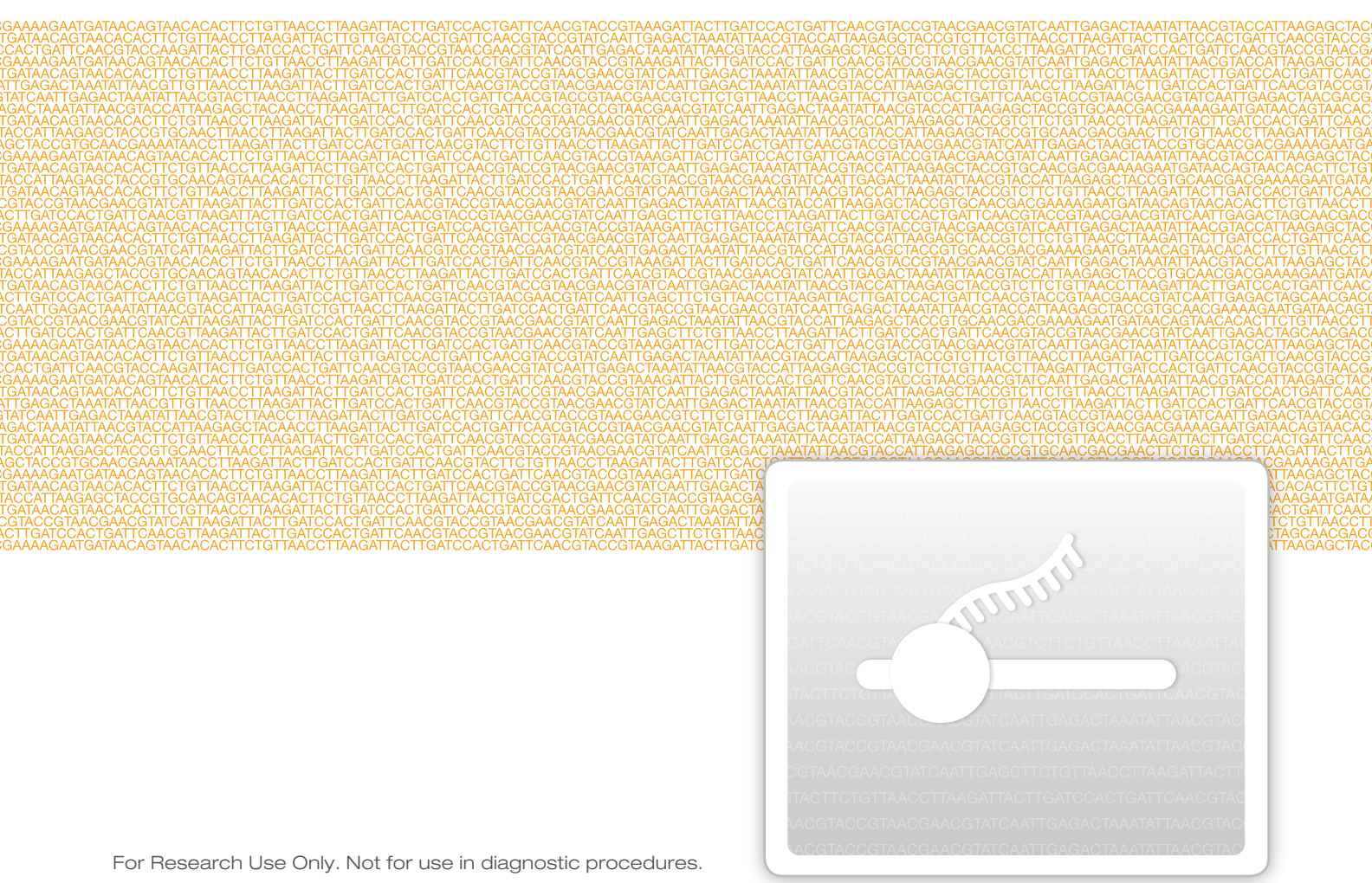
Ribo-Zero® rRNA Removal Kit Reference Guide



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Document # 15066012 v02
August 2016

Customize a short end-to-end workflow guide with the Custom Protocol Selector
support.illumina.com/custom-protocol-selector.html

Revision History

Document	Date	Description of Change
Document # 15066012 v02	August 2016	• Updated the Remove rRNA procedure to specify the volume of washed magnetic beads that RNA sample is added to, and to clarify the volume of supernatant transferred to a new tube. • Updated terminology to use the term sample instead of reaction. • Added consumables list to the Remove rRNA step. • Removed input recommendations and protocol steps for low input samples. • Corrected the volume of sample required for the Hybridize Probes step to 8–10 µl. • Corrected name of beads provided in the Agencourt RNAClean XP kit to RNAClean XP beads.
Document # 15066012 v01	July 2016	• Renamed this guide to *Ribo-Zero rRNA Removal Kit Reference Guide*, which includes the full kit name and reflects that this guide is a reference guide. • Updated kit features, including correcting input requirements from 1 µg total RNA to ≥ 100 ng total RNA. • Specified water grade for diluting total RNA samples, and removed TE buffer as a diluent. • Updated design of workflow diagram. • Added sections containing additional resources, tips and techniques, and acronyms. • Renamed and combined some procedures as needed to improve continuity. • Simplified consumables information at the beginning of each section. • Revised step-by-step instructions to be more succinct. • Added protocol steps for low input samples. • Clarified methods for RNA quantification and assessing yield and quality. • Added kit catalog numbers and reagent quantities. • Corrected ratio of AMPure XP beads from 1.6x to 1.8x. • Moved troubleshooting information and performance specifications to the web.
Document # 15066012 Rev. A	November 2014	Initial release.

Table of Contents

Overview

Chapter 1

Introduction

This protocol explains how to use a Ribo-Zero® rRNA Removal Kit to remove rRNA from total RNA. The goal of this protocol is to wash and resuspend magnetic beads, which then bind to removal probes hybridized to rRNA, producing an RNA sample ready for library prep.

The Ribo-Zero protocol offers:

- Efficient removal of rRNA from samples.
- Downstream sequencing data that contains complete transcriptome of coding and noncoding RNA species.
- Support for intact and partially degraded small samples, requiring input of ≥ 1 µg total RNA.

RNA Input Recommendations

The Ribo-Zero rRNA Removal Kit supports rRNA depletion from 1–5 µg total RNA samples. For the Epidemiology kit, input of 500 ng–2.5 µg and total RNA samples is supported.

DNA-Free RNA

The RNA samples must be free of contaminating DNA, which can cause inaccurate RNA quantification, interfere with rRNA removal, and negatively affect library prep and sequencing.

Before beginning the Ribo-Zero protocol, treat the sample with Baseline-ZERO DNase and then purify the treated RNA.

RNA Quantification

Use a fluorometric method to quantify the amount of total RNA in the sample. Accurate RNA quantification is necessary for determining the volume of removal solution and the maximum volume in which the total RNA sample can be dissolved when preparing to treat it with removal solution.

Dilute the sample in molecular biology-grade water. Do not exceed the recommended input amount of total RNA, which is:

- 5 µg for most Ribo-Zero kits.
- 2.5 µg for the Ribo-Zero (Epidemiology) kit.

Compare rRNA content before and after a Ribo-Zero rRNA Removal Kit reaction to assess the depletion of rRNA in total RNA. The success of rRNA removal depends on factors such as species, sample quality, and sequence homology between the rRNA sample and removal solution, especially with 5S rRNA removal. Use the RNAMatchMaker at epibio.com/rnamatchmaker to verify sample and kit sequence homology sequence homology.

FFPE-RNA Samples

You can use RNA extracted from highly degraded total RNA, such as that obtained from formalin-fixed paraffin-embedded (FFPE), tissue with Ribo-Zero kits. However, the quality of FFPE RNA is highly variable due to the tissue-fixation procedure, sample age, storage conditions, fixation reversal process, and other issues. Therefore, Illumina® cannot guarantee success with every FFPE RNA sample.

Additional Resources

Visit the Ribo-Zero rRNA Removal Kit support page on the Illumina website for documentation, software downloads, training resources, and information about compatible Illumina products.

Protocol

Introduction

This chapter describes the Ribo-Zero rRNA Removal Kit protocol.

- Before proceeding, confirm kit contents and make sure that you have the required equipment and consumables. See *Supporting Information* on page 21.
- Follow the protocols in the order shown, using the specified volumes and incubation parameters.

Tips and Techniques

Unless a safe stopping point is specified in the protocol, proceed immediately to the next step.

Core Kit Temperature

- ***Do not freeze the core kit or put the contents on ice.*** Freezing the magnetic beads damages them, decreasing kit performance.
- Always use the magnetic beads at room temperature. Store at 2°C to 8°C when not in use.

Automation

- Illumina supports Illumina liquid handling robotic platforms and protocols only. Contact the manufacturer of your liquid handling instrument for hardware, software, protocol, and technical support.
- When using an automated liquid handling instrument with magnetic beads, do not use conductive tips.

Avoiding Cross-Contamination

- When adding or transferring samples, change tips between ***each sample***.
- When adding adapters or primers, change tips between ***each tube***.

Capping the Tubes

- Always cap the tubes before the following steps in the protocol:
  - Shaking steps
  - Vortexing steps
  - Centrifuge steps
  - Thermal cycling steps

Tube Transfers

- When transferring volumes between tube strips, transfer the specified volume from each tube of a strip to the corresponding tube of the other strip.
- If beads are aspirated into the pipette tips, dispense the sample back into the tube on the magnetic stand and wait until the liquid is clear (~2 minutes).

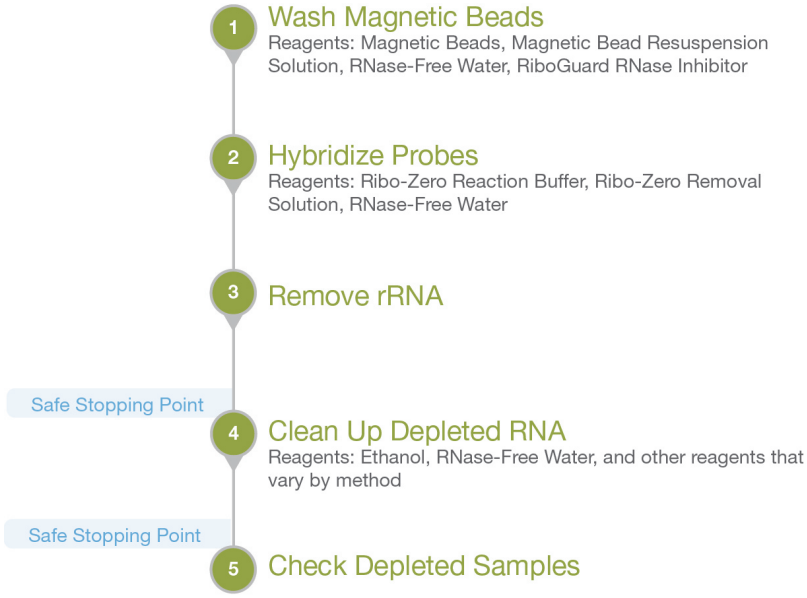
Centrifugation

- Centrifuge at any step in the procedure to consolidate liquid or beads in the bottom of the tube, and to prevent sample loss.

RNA Removal Worfklow

The following figure illustrates the Ribo-Zero workflow. Safe stopping points are marked between steps.

Figure 1 Ribo-Zero Workflow



Wash Magnetic Beads

Wash the magnetic beads using 1 of 2 methods:

- Individual washing for 6 or fewer samples, as described in *Individual Washing* on page 9.
- Batch washing for 7 or more samples, as described in *Batch Washing* on page 10.

Individual Washing

Consumables

- 1.5 ml RNase-free microcentrifuge tubes with caps
- Magnetic beads (225 µl per sample)
- RNase-free water (225 µl per sample)
- Magnetic Bead Resuspension Solution (65 µl per sample)
- [Optional] RiboGuard RNase Inhibitor (1 µl per sample)

About Reagents

- Dispense magnetic beads slowly to avoid air bubbles.
- RiboGuard RNase Inhibitor is recommended to prevent RNA degradation from RNase contaminants.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
Magnetic beads	2°C to 8°C	Bring to room temperature. Vortex to mix.
Magnetic Bead Resuspension Solution	2°C to 8°C	Bring to room temperature.
RNase-free water	2°C to 8°C	Thaw on ice. Invert to mix, and then centrifuge briefly.
[Optional] RiboGuard RNase Inhibitor	-85°C to -65°C	Thaw on ice. Invert to mix, and then centrifuge briefly.

Procedure

1 For each reaction, add 225 µl magnetic beads to a 1.5 ml microcentrifuge tube.

2 Place on a magnetic stand, with cap open, and wait until the liquid is clear (~1 minute).

3 Remove and discard all supernatant.

CAUTION
The supernatant contains 0.1% sodium azide.

4 Remove from the magnetic stand.

5 Wash 2 times as follows.

a Add 225 µl RNase-free water.
b Vortex to resuspend.

6 Place on a magnetic stand, with cap open, and wait until the liquid is clear (~1 minute).

7 Remove and discard all supernatant.

8 Remove from the magnetic stand.

9 Add 65 µl Magnetic Bead Resuspension Solution.

10 Vortex to resuspend.

11 [Optional] Add 1 µl RiboGuard RNase Inhibitor, and then pipette to mix.

12 Set aside at room temperature.

Batch Washing

Consumables

- RNase-free tubes with caps
  - 1.5 ml microcentrifuge tube to wash up to 1350 µl magnetic beads (up to 6 samples)
  - 15 ml centrifuge tube to wash more than 1350 µl magnetic beads (7 or more samples)
- Magnetic beads (225 µl per sample)
- RNase-free water (225 µl per sample)
- Magnetic Bead Resuspension Solution (60 µl per sample)
- [Optional] RiboGuard RNase Inhibitor (1 µl per sample)

About Reagents

- 1350 µl magnetic beads is sufficient for 6 samples. You can divide the magnetic beads among multiple 1.5 ml tubes, or wash them all in a 15 ml tube.
- Dispense magnetic beads slowly to avoid air bubbles.
- RiboGuard RNase Inhibitor is recommended to prevent RNA degradation from RNase contaminants.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
Magnetic beads	2°C to 8°C	Bring to room temperature. Vortex to mix.
Magnetic Bead Resuspension Solution	2°C to 8°C	Bring to room temperature.
RNase-free water	2°C to 8°C	Thaw on ice. Invert to mix, and then centrifuge briefly.
[Optional] RiboGuard RNase Inhibitor	-85°C to -65°C	Thaw on ice. Invert to mix, and then centrifuge briefly.

Procedure

1 For each sample, add 225 µl magnetic beads to a 1.5 ml microcentrifuge tube or 15 ml centrifuge tube.

2 Place on a magnetic stand, with cap open, and wait until the liquid is clear (~1 minute).

3 Remove and discard all supernatant.

CAUTION
The supernatant contains 0.1% sodium azide.

4 Remove from the magnetic stand.

5 Wash 2 times as follows.

   a Add 225 µl RNase-free water.
   b Vortex to resuspend.

6 Place on a magnetic stand, with cap open, and wait until the liquid is clear (~1 minute).

7 Remove and discard all supernatant.

8 Remove from the magnetic stand.

9 Add 60 µl Magnetic Bead Resuspension Solution.

10 Vortex to resuspend.

11 Add 65 µl magnetic beads per sample to a fresh 1.5 ml tube.

12 [Optional] Add 1 µl RiboGuard RNase Inhibitor to each tube. Vortex briefly to mix.

13 Set aside at room temperature.

Hybridize Probes

In this step, probes in the removal solution hybridize to rRNA present in the sample. Before probe hybridization, the sample must be purified and free of gDNA contamination.

Consumables

- 0.2 ml or 0.5 ml RNase-free microcentrifuge tubes with caps
- RNase-free water
- Ribo-Zero Reaction Buffer (4 µl per sample)
- Ribo-Zero Removal Solution (8–10 µl per sample)

About Reagents

- Thorough mixing is important at this step.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
Ribo-Zero Reaction Buffer	-85°C to -65°C	Thaw on ice. Invert to mix, and then centrifuge briefly.
Ribo-Zero Removal Solution	-85°C to -65°C	Thaw on ice. Invert to mix, and then centrifuge briefly.
RNase-free water	2°C to 8°C	Thaw on ice. Invert to mix, and then centrifuge briefly.

2 Set a heat block or thermal cycler to 68°C.

Procedure

1 Use the following table to determine the appropriate volumes of RNA sample and Ribo-Zero Removal Solution for use in step 2.

Table 1 RNA and Reagent Volumes

Total RNA Input for Epidemiology Kit	Total RNA Input for Other Ribo-Zero Kits	Max Total RNA per Sample	Removal Solution per Sample
500 ng to 1.25 µg	1–2.5 µg	28 µl	8 µl
> 1.25–2.5 µg	> 2.5–5 µg	26 µl	10 µl

2 For each sample, combine the following volumes in a 0.2 ml or 0.5 ml microcentrifuge tube. Pipette to mix.
   - RNase-free water (x µl)
   - Ribo-Zero Reaction Buffer (4 µl)
   - RNA sample (y µl)
   - Ribo-Zero Removal Solution (8–10 µl)

   The total volume per sample is 40 µl.

3 Place on the preheated heat block or thermal cycler and incubate for 10 minutes.

4 Remove from heat, and then centrifuge briefly.

5 Incubate at room temperature for 5 minutes.

Remove rRNA

This step combines the probe-hybridized samples with the washed magnetic beads, which bind to the probes. The recovered RNA sample is depleted of rRNA.

Consumables

- 1.5 ml RNase-free microcentrifuge tubes with caps

Preparation

1. Set a heat block or thermal cycler to 50°C.

Procedure

1. For each sample, do as follows.
   a. Add 40 µl RNA sample to a 1.5 ml tube containing 65 µl washed magnetic beads. ***Immediately*** pipette to mix.
   b. Vortex for 10 seconds, and then set aside at room temperature.
2. Incubate at room temperature for 5 minutes.
3. Place on the preheated heat block or thermal cycler and incubate for 5 minutes.
4. Immediately place on a magnetic stand, with cap open, and wait until the liquid is clear (~1 minute).
5. Transfer 85–90 µl supernatant containing depleted RNA to a fresh 1.5 ml tube.
6. Set aside on ice.

SAFE STOPPING POINT

If you are stopping, cap the tubes and store at -25°C to -15°C overnight or at -85°C to -65°C for up to 30 days.

Clean Up Depleted RNA

RNA samples depleted of rRNA must be purified before library prep. The cleanup step removes any remaining salts and buffers and concentrates the depleted samples.

NOTE
A high proportion of small RNA in samples treated with Ribo-Zero is normal does not indicate degraded RNA.

Clean up the depleted RNA using 1 of the following methods:

Cleanup Method	Description
Ethanol precipitation *Ethanol Precipitation* on page 14	Provides optimal recovery of small RNA, such as miRNA and tRNA, with mRNA and large noncoding RNA.
Agencourt RNAClean XP *Agencourt RNAClean XP Kit* on page 15	Uses the Beckman Coulter Agencourt RNAClean XP kit to recover RNA. This method does not recover small RNA quantitatively.
Modified RNeasy MinElute Cleanup Kit *Modified RNeasy MinElute Cleanup Kit* on page 16	Modifies manufacturer instructions for the QIAGEN RNeasy MinElute Cleanup Kit for use with the Ribo-Zero protocol. This method provides optimal recovery of large and small RNA.
RNA Clean & Concentrator-5 *RNA Clean & Concentrator-5 Kit* on page 18	Uses the Zymo Research RNA Clean & Concentrator-5 kit to recover large and small RNA, or only large (> 200 nt) RNA.

Ethanol Precipitation

Consumables

- 1.5 ml RNase-free microcentrifuge tubes with caps
- Freshly prepared and ice-cold 70% (v/v) ethanol
- Ice-cold 100% ethanol
- 3 M sodium acetate (18 µl per sample)
- 10 mg/ml glycogen (2 µl per sample)
- RNase-free water (188.5–190 µl per sample)

About Reagents

- Always prepare fresh 70% ethanol. Ethanol can absorb water from the air, impacting results.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
3 M sodium acetate	-85°C to -65°C	Thaw on ice. Invert to mix, and then centrifuge briefly.

Item	Storage	Instructions
10 mg/ml glycogen	-85°C to -65°C	Thaw on ice. Invert to mix, and then centrifuge briefly.
RNase-free water	-85°C to -65°C	Thaw on ice. Invert to mix, and then centrifuge briefly.

2 Prepare fresh 70% ethanol from absolute ethanol.

3 Set aside both forms of ethanol on ice.

Procedure

Complete the following procedure for each tube of depleted sample.

1 Add RNase-free water to bring the volume to 180 µl.

2 Add 18 µl 3 M sodium acetate.

3 Add 2 µl glycogen (10 mg/ml).

4 Vortex to mix.

5 Add 600 µl 100% ethanol.

6 Vortex to mix.

7 Set aside at -25°C to -15°C for at least 1 hour.

8 Centrifuge at 10,000 × g for 30 minutes at 4°C.

9 Remove and discard all supernatant.

10 Wash 2 times as follows.

   a Add 200 µl freshly prepared 70% ethanol.
   b Centrifuge at 10,000 × g for 5 minutes at 4°C.
   c Remove and discard all supernatant.

11 Centrifuge briefly to collect any residual supernatant.

12 Remove and discard all supernatant.

13 Dry at room temperature for 5 minutes.

14 Dissolve the pellet in the appropriate amount of RNase-free water or buffer for your library prep kit:
   - **ScriptSeq**—10 µl
   - **TruSeq Stranded mRNA**—8.5 µl

SAFE STOPPING POINT

If you are stopping, cap the tubes and store at -25°C to -15°C overnight or at -85°C to -65°C for up to 30 days.

Agencourt RNAClean XP Kit

Consumables

- 0.2 ml or 1.5 ml RNase-free microcentrifuge tubes with caps
- Beckman Coulter Agencourt RNAClean XP kit
- Freshly prepared and ice-cold 80% (v/v) ethanol
- RNase-free water (9.5–11 µl per reaction)

About Reagents

- Vortex RNAClean XP beads before each use.
- Vortex RNAClean XP beads frequently to make sure that the beads are even distributed.
- Always prepare fresh 80% ethanol. Ethanol can absorb water from the air, impacting results.

Preparation

1 Prepare the following consumables:

Item	Storage	Instructions
RNase-free water	-85°C to -65°C	Thaw on ice. Invert to mix, and then centrifuge briefly.

2 Prepare fresh 80% ethanol from absolute ethanol.

3 Set aside both forms of ethanol on ice.

4 Follow the manufacturer instructions to prepare consumables in the Agencourt RNAClean XP kit.

Procedure

1 Follow the manufacturer instructions to purify the depleted RNA samples with the Agencourt RNAClean XP kit. Use 160 µl (1.8x) RNAClean XP beads per tube containing 85–90 µl sample.

Modified RNeasy MinElute Cleanup Kit

Consumables

- 1.5 ml RNase-free microcentrifuge tubes with caps
- QIAGEN RNeasy MinElute Cleanup Kit
- Ice-cold 96% (v/v) to 100% ethanol (550 µl per sample)
- Freshly prepared and ice-cold 80% (v/v) ethanol (550 µl per sample)
- RNase-free water

About Reagents

- Always prepare fresh 80% ethanol. Ethanol can absorb water from the air, impacting results.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
RNase-free water	-85°C to -65°C	Thaw on ice. Invert to mix, and then centrifuge briefly.

2 Prepare fresh 80% ethanol from absolute ethanol.

3 Set aside both forms of ethanol on ice.

4 Follow the manufacturer instructions to prepare consumables in the RNeasy MinElute Cleanup Kit. Make sure that ethanol is added to Buffer RPE before use.

Procedure

Complete the following procedure for each tube of depleted sample.

1 Add RNase-free water to bring the volume to 100 µl.

2 Add 350 µl Buffer RLT. Pipette to mix.

3 Add 550 µl 96%–100% ethanol. Pipette to mix.

4 Add half the sample mixture (~500 µl) to an RNeasy MinElute spin column placed in a 2 ml collection tube.

5 Close the lid, and then centrifuge at 8000 × g for 15 seconds.

6 Discard the flow-through.

7 Add the remaining sample to the spin column.

8 Repeat steps 4–6.

9 Discard the flow-through and collection tube.

10 Place the RNeasy MinElute spin column in a new 2 ml collection tube.

11 Add 500 µl Buffer RPE, and then close the lid.

12 Centrifuge at 8000 × g for 15 seconds.

13 Discard the flow-through and retain the collection tube.

14 Add 500 µl 80% ethanol to the RNeasy MinElute spin column, and then close the lid.

15 Centrifuge at 8000 × g for 2 minutes.

16 Without allowing the column to contact the flow-through, remove the RNeasy MinElute spin column from the collection tube.
Contact between the column and flow-through causes ethanol carryover.

17 Discard the flow-through and collection tube.

18 Place the RNeasy MinElute spin column in a new 2 ml collection tube.

19 Place the spin columns into the centrifuge with at least 1 empty position between columns, which avoids damaging the spin column lids. Orient the lids to point in a direction opposite the rotation of the rotor.
For example, if the rotor rotates clockwise, orient the lids counterclockwise.

20 With the lids open, centrifuge at maximum speed for 5 minutes.
Centrifugation with an open lid prevents ethanol carry over during RNA elution and dries the spin column membrane. Drying is important because residual ethanol can interfere with downstream applications.

21 Discard the flow-through and collection tube.

22 Place the RNeasy MinElute spin column in a new 1.5 ml collection tube.

23 Add the appropriate amount of RNase-free water or buffer for your library prep kit to the center of the spin-column membrane:
- **ScriptSeq**—10 µl
- **TruSeq Stranded mRNA**—8.5 µl

24 Close the lid, and then centrifuge at maximum speed for 1 minute.
Expect to recover ~10 µl RNA.

SAFE STOPPING POINT

If you are stopping, cap the tubes and store at -25°C to -15°C overnight or at -85°C to -65°C for up to 30 days.

RNA Clean & Concentrator-5 Kit

Consumables

- 1.5 ml RNase-free microcentrifuge tubes with caps
- Zymo Research RNA Clean & Concentrator-5 kit
- Ice-cold 96% (v/v) to 100% ethanol
- Freshly prepared ice-cold 80% (v/v) ethanol
- RNase-free water

About Reagents

- Always prepare fresh 80% ethanol. Ethanol can absorb water from the air, impacting results.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
RNase-free water	-85°C to -65°C	Thaw on ice. Invert to mix, and then centrifuge briefly.

2 Prepare fresh 80% ethanol from absolute ethanol.

3 Set aside both forms of ethanol on ice.

Procedure

1 Follow the manufacturer instructions to purify RNA > 17 nt or RNA > 200 nt.

SAFE STOPPING POINT

If you are stopping, cap the tubes and store at -25°C to -15°C overnight or at -85°C to -65°C for up to 30 days.

Check Depleted Samples

Perform the following procedures to check the quality and quantity of depleted RNA samples.

Assess Yield

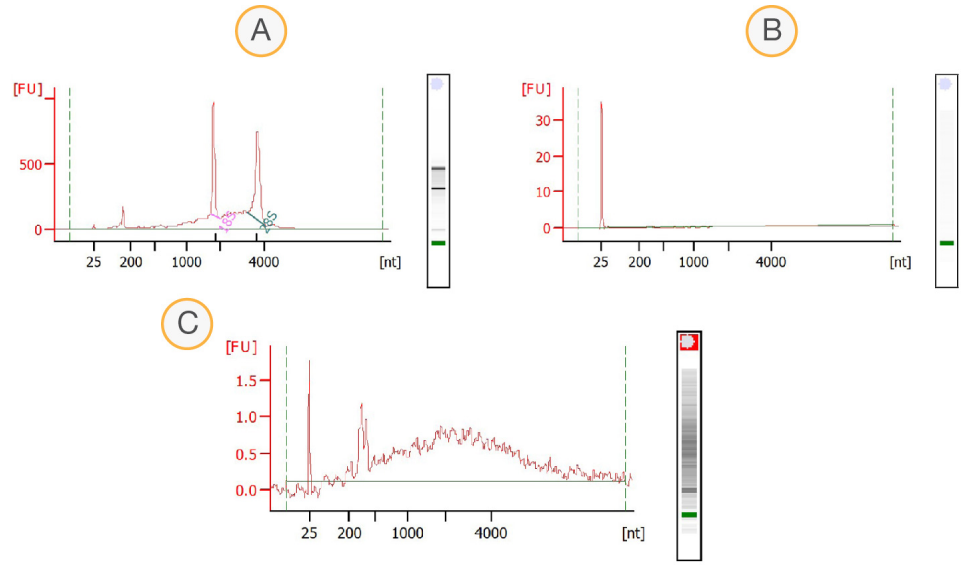
The yield of depleted RNA depends on the amount of input total RNA, the rRNA content of the sample, and the cleanup method. For example, input of 1 µg total RNA yields < 80 ng RNA depleted of rRNA.

1 Quantify the yield of the RNA treated with Ribo-Zero using a fluorometric method. Expect to recover < 8% of the amount of input RNA.

Assess Quality

1 Run 1 µl Ribo-Zero-treated RNA on an Agilent 2100 Bioanalyzer using an RNA 6000 Pico Chip.
The Agilent RNA Nano Chip does not provide sufficient sensitivity.

Figure 2 Typical Depletion Traces Using the Agilent RNA 6000 Pico Chip



A Universal Human Reference Total RNA diluted to 25 ng/µl (1 µl loaded).

B 1 µg of Universal Human Reference Total RNA after treatment with the Ribo-Zero rRNA Removal Kit (Human/Mouse/Rat). Note the scale in the traces. Results are similar with other organisms.

C The results from B, magnified ~20×.

Additional Validation (Optional)

1 To make sure that rRNA is removed, create RT-qPCR primers to the sample species rRNA with a housekeeping gene.

NOTE
This option is recommended.

Supporting Information

Appendix A

Introduction

The protocols described in this guide assume that you have reviewed the contents of this appendix, confirmed your kit contents, and obtained all the required consumables and equipment.

Acronyms

Acronym	Definition
FFPE	Formalin-Fixed Paraffin-Embedded
mRNA	Messenger RNA
miRNA	Micro RNA
rRNA	Ribosomal RNA
tRNA	Transfer RNA

Kit Contents

Make sure that you have all reagents identified in this section before proceeding to the library preparation procedures. Ribo-Zero kits are successful with various species, and are available in the following configurations.

Table 2 Ribo-Zero Kits

Kit Name	Catalog #
Ribo-Zero Gold rRNA Removal Kit (Epidemiology) 6 reactions	MRZE706
Ribo-Zero Gold rRNA Removal Kit (Epidemiology) 24 reactions	MRZE724
Ribo-Zero Gold rRNA Removal Kit (Human/Mouse/Rat) 6 reactions	MRZG126
Ribo-Zero Gold rRNA Removal Kit (Human/Mouse/Rat) 24 reactions	MRZG12324
Ribo-Zero Gold rRNA Removal Kit (Yeast) 6 reactions	MRZY1306
Ribo-Zero Gold rRNA Removal Kit (Yeast) 24 reactions	MRZY1324
Ribo-Zero rRNA Removal Kit (Bacteria) 6 reactions	MRZMB126
Ribo-Zero rRNA Removal Kit (Bacteria) 24 reactions	MRZB12424
Ribo-Zero rRNA Removal Kit (Gram-Negative Bacteria) 6 reactions	MRZGN126
Ribo-Zero rRNA Removal Kit (Gram-Positive Bacteria) 6 reactions	MRZGP126
Ribo-Zero rRNA Removal Kit (Human/Mouse/Rat) 6 reactions	MRZH116
Ribo-Zero rRNA Removal Kit (Human/Mouse/Rat) 24 reactions	MRZH11124
Ribo-Zero rRNA Removal Kit (Plant Leaf) 6 reactions	MRZPL116
Ribo-Zero rRNA Removal Kit (Plant Leaf) 24 reactions	MRZPL1224
Ribo-Zero rRNA Removal Kit (Plant Seed/Root) 6 reactions	MRZSR116

Each kit contains Ribo-Zero reagents and a core kit. Reagent quantities are the same for each kit, but the volumes vary by the number of reactions.

Ribo-Zero Reagents, Store at -85°C to -65°C

Quantity	Reagent
1	RiboGuard RNase Inhibitor
1	Ribo-Zero Reaction Buffer
1	Ribo-Zero Removal Solution
2	RNase-Free Water
1	Glycogen (10 mg/ml)
1	Sodium Acetate (3 M)

Core Kit, Store at 2°C to 8°C

Quantity	Component Name
1	Magnetic Beads
1	Magnetic Bead Resuspension Solution
2	RNase-Free Water

Library Prep Kits

The Ribo-Zero rRNA Removal Kit is designed to remove rRNA and does not include library prep reagents. After completing the Ribo-Zero protocol, prepare libraries using any Illumina library prep kit that is RNA-compatible.

Table 3 Options for Library Prep

Library Prep Kit	Includes Ribo-Zero Reagents	Requires Stand-Alone Ribo-Zero Kit
ScriptSeq v2	No	Yes
TruSeq Stranded mRNA	No	Yes
ScriptSeq Complete Gold Kit*	Yes	No
TruSeq Stranded Total RNA Kit with Ribo-Zero Gold*	Yes	No

*These kits provide options for bundling Ribo-Zero with library prep. For catalog numbers, see the product support page.

Consumables and Equipment

Make sure that you have the required user-supplied consumables and equipment before starting the protocol.

Consumables

Consumable	Supplier
0.2 ml or 0.5 ml RNase-free microcentrifuge tubes with caps	General lab supplier
1.5 ml RNase-free microcentrifuge tubes with caps	General lab supplier
Absolute (100%) ethanol	General lab supplier
Baseline-ZERO DNase	Epicentre, catalog # DB0711K or DB0715K
An RNA purification kit: • RNeasy MinElute Cleanup Kit • Agencourt RNAClean XP, 40 ml kit • RNA Clean & Concentrator-5	One of the following suppliers, depending on kit selection: • QIAGEN, catalog # 74204 • Beckman Coulter, catalog # A63987 • Zymo Research, catalog # R1015 or R1016
[Optional] 15 ml RNase-free centrifuge tubes with caps	General lab supplier

Equipment

Equipment	Supplier
1.5 ml microcentrifuge	General lab supplier
Magnetic rack or stand for 1.5 ml tubes	Bangs Laboratories, Inc., catalog # LS001, MS002, or MS003 Life Technologies, catalog # 12321D
Thermal cycler or heat block	General lab supplier
Vortexer	General lab supplier
[Optional] Magnetic rack or stand for 15 ml tubes	Millipore, catalog # LSKMAGS15

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 4 Illumina General Contact Information

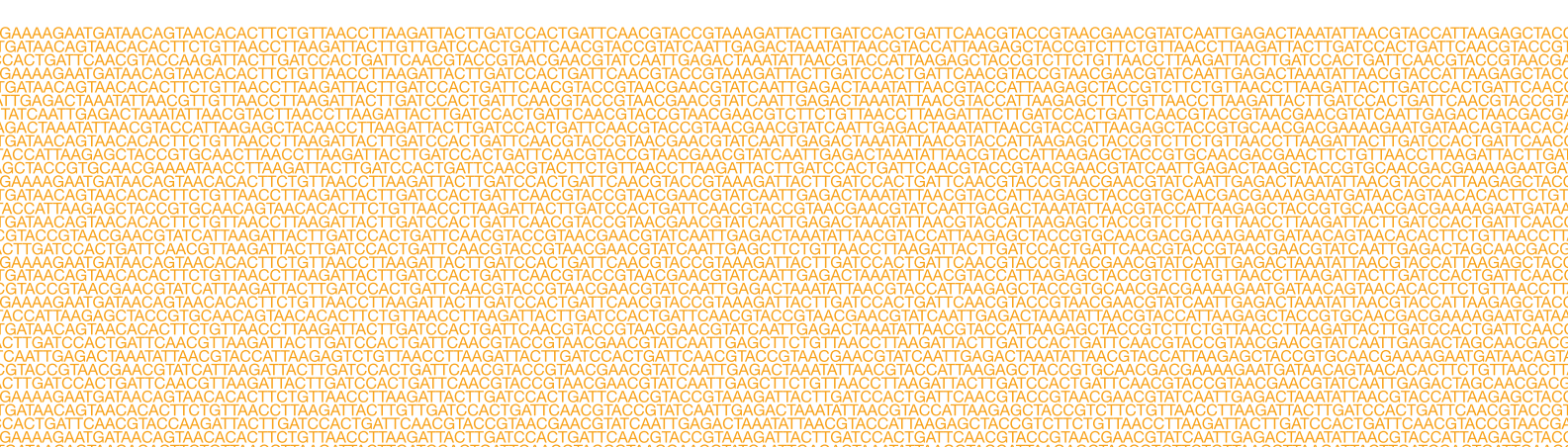
Website	www.illumina.com
Email	techsupport@illumina.com

Table 5 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.



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