Ribo-Zero™ Magnetic Kit* Small

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Ribo-Zero™ Magnetic Kit*

(Human/Mouse/Rat)

Cat. No. MRZH116 – 6 Reactions
(Contains 1 box of Cat. No. RZH1046 and
1 box of Cat. No. MRZ116C)

Cat. No. MRZH11124 – 24 Reactions
(Contains 1 box of Cat. No. RZH110424 and
1 box of Cat. No. MRZ11124C)

** Patent Pending*

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Quick Protocol for Ribo-Zero™ Magnetic Kit (Human/Mouse/Rat)

For experienced users only!

Step	Procedure	Pages
Prepare Magnetic Beads	Add 225 µl Magnetic Beads to RNase-free tube. Magnetize for 1 min at RT. Wash with 225 µl RNase-Free Water. Magnetize, repeat wash step. Resuspend in 65 µl Resuspension Solution Optional: Add 1 µl RiboGuard RNase Inhibitor	4
Treat sample with rRNA Removal Solution	Mix in 40 µl total volume: 1-5 µg total RNA 8-10 µl rRNA Removal Solution 4 µl Reaction Buffer Incubate 10 min @ 68˚C, then 5 min @ RT	6
Remove rRNA	Mix previously prepared Magnetic Beads Add RNA mixture, mix well by pipetting, vortex briefly Incubate 5 min @ RT, vortex Incubate 5 min @ 50˚C Magnetize, transfer supernatant (rRNA-depleted sample) to RNase-free tube	7
Purify rRNA-depleted sample	Ethanol precipitation or alternative method	7

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1. Kit Contents and Specifications

The kit components are supplied in tubes with colored caps for easier identification. Each kit contains one box of Cat. No. RZH1046/RZH110424 and one box of Cat. No. MRZ116C/MRZ11124C.

Ribo-Zero™ rRNA Removal Kit (Human/Mouse/Rat) (Cat. No. RZH1046/RZH110424)

Component Name	Tube Label	Volume		Cap Color
		6 rxn	24 rxn	
RiboGuard RNase Inhibitor (100 U/µl)	RiboGuard RNase Inhibitor	10 µl	30 µl	Blue
Ribo-Zero rRNA Removal Solution (Human/Mouse/Rat)	rRNA Removal Solution-(H/M/R)	75 µl	270 µl	
Ribo-Zero Reaction Buffer	Ribo-Zero Reaction Buffer	50 µl	110 µl	
Glycogen (10 mg/ml)	Glycogen	20 µl	60 µl	Clear
Sodium Acetate (3 M)	Sodium Acetate	150 µl	500 µl	
RNase-Free Water	RNase-Free Water	2 x 1 ml	3 x 1.5 ml	

Storage: Store this kit box and its contents at –70°C to –80°C.

Magnetic Core Kit (Cat. No. MRZ116C/MRZ11124C)

Component Name	Tube Label	Volume		Cap Color
		6 rxn	24 rxn	
Magnetic Beads	Magnetic Beads	1.4 ml	5.4 ml	Clear
Magnetic Bead Resuspension Solution	Magnetic Bead Resuspension Solution	500 µl	2.0 ml	Yellow/ Clear
RNase-Free Water	RNase-Free Water	2 x 1.5 ml	11 ml	Clear

Storage: Store this kit box and its contents at 4°C (Do Not Freeze!).

Additional Required Reagents and Equipment:

Magnetic rack or stand
0.2-ml or 0.5-ml microcentrifuge tubes (RNase-free)
Thermocycler or other temperature control device for 0.2-ml or 0.5-ml tubes
Water bath, heating block, or other temperature control device for 1.5-ml tubes
Microcentrifuge
Ice-cold 100% and 70% ethanol
Optional: RNA purification kits

Performance Specifications and Quality Control

A Ribo-Zero Magnetic Kit (Human/Mouse/Rat) reaction removes >98% of 28S, 18S, and 5.8S and >95% of 5S rRNAs from intact Human Reference RNA as assessed by qRT-PCR before and after a Ribo-Zero Magnetic Kit reaction.

2. RNA Sample Considerations

DNA-Free RNA

Treat the RNA sample with DNase I to remove all traces of DNA. Then, remove the DNase I prior to the Ribo-Zero reaction. DNA contamination will interfere with rRNA removal and is the main cause of loss of directionality when sequencing RNA-Seq libraries. The RNA sample should be free of salts (e.g., Mg^{2+} or guanidinium salts) or organics (e.g., phenol and ethanol).

Amount of RNA

A kit reaction uses 1-5 µg of input total RNA. The sample should be dissolved in RNase-Free Water or TE Buffer. It is important to quantify the amount of total RNA in the sample as accurately as possible in order to use the appropriate amount of Ribo-Zero rRNA Removal Solution in Part 3.B. Use Table 1 (Part 3.B) to determine the maximum volume in which the total RNA sample can be dissolved prior to performing the Ribo-Zero procedure.

Yield of Ribo-Zero-Treated RNA

Typically 3%-10% of the input total RNA Is recovered from a Ribo-Zero procedure. The yield is dependent on the amount of input total RNA, the rRNA content of the sample, and the method used to purify the Ribo-Zero-treated RNA.

3. Kit Procedure

3.A. Preparation of the Magnetic Beads

Required in Part 3.A

Component Name	Tube Label	Cap Color
Magnetic Beads	Magnetic Beads	Clear
RNase-Free Water	RNase-Free Water	
Magnetic Bead Resuspension Solution	Magnetic Bead Resuspension Solution	Yellow/Clear
RiboGuard RNase Inhibitor (100 U/µl)	RiboGuard RNase Inhibitor	Blue

Important! *Allow Magnetic Core Kit components to equilibrate to room temperature for use in Step 2.*

Remove the Ribo-Zero rRNA Removal Kit from –70°C to –80°C storage, thaw the tubes, and place them on ice. Wash the beads by using either the batch washing or individual washing procedure.

3.A.1. Batch Washing Procedure

1. For each Ribo-Zero reaction, 225 µl of the Magnetic Beads is required.

   Note: *Mix the Magnetic Beads well by pipetting or gentle vortexing.*

2. Determine the amount of Magnetic Beads required for the total number of reactions and dispense a maximum of 1,350 µl into each 1.5-ml RNase-free microcentrifuge tube (sufficient for six reactions). Pipet the Magnetic Bead suspension *slowly* to

avoid air bubbles and to ensure pipetting of the correct volume. Store unused Magnetic Beads at 4°C.

Note: *When setting up more than six Ribo-Zero reactions, either multiples of 1,350-µl aliquots can be washed in a RNase-free 1.5 ml microcentrifuge tubes, or a larger volume can be washed in RNase-free 15-ml tubes (e.g., using a 15-ml magnetic stand).*

3. Place the 1.5-ml microcentrifuge tube containing the Magnetics Beads on the magnetic stand for at least 1 minute (until the solution appears clear).
4. With the 1.5-ml microcentrifuge tube still on the stand, remove and discard the supernatant.

⚠ ***Caution:*** *The supernatant contains 0.1% sodium azide.*

5. Remove the 1.5-ml microcentrifuge tube from the stand and add an equal volume of RNase-Free Water. Mix well by repeated pipetting or by vortexing at medium speed.
6. Repeat Steps 3 and 4.
7. Remove the 1.5-ml microcentrifuge tube from the magnetic stand. Add a volume of Magnetic Bead Resuspension Solution equal to the number of reactions x 60 µl (e.g., for 6 reactions, add 6 x 60 µl = 360 µl Magnetic Bead Resuspension Solution). Mix well by repeated pipetting or by vortexing at medium speed.

   Note: *The volumes of the beads and Resuspension Solution are additive. Although the washed beads are resuspended in 60 µl per reaction, each reaction uses 65 µl of resuspended beads.*

8. Aliquot 65 µl of the washed Magnetic Beads into each new 1.5-ml RNase-free microcentrifuge tube (corresponding to the number of Ribo-Zero reactions).
9. Optional: Add 1 µl of RiboGuard RNase Inhibitor to each tube of resuspended Magnetic Beads, and mix briefly by vortexing.
10. Store the microcentrifuge tubes at room temperature until required in Part 3.C.

3.A.2. Individual Washing Procedure

Note: *Mix the Magnetic Beads well by pipetting or gentle vortexing.*

1. For each reaction, pipet 225 µl of Magnetic Beads into a 1.5-ml RNase-free microcentrifuge tube. Pipet the Magnetic Bead suspension *slowly* to avoid air bubbles and to ensure pipetting of the correct volume. Store unused Magnetic Beads at 4°C.
2. Place each 1.5-ml microcentrifuge tube on the magnetic stand for at least 1 minute (until the solution appears clear).
3. With the 1.5-ml microcentrifuge tube still on the stand, remove and discard the supernatant.

⚠ ***Caution:*** *The supernatant contains 0.1% sodium azide.*

4. Remove the 1.5-ml microcentrifuge tube from the magnetic stand and add 225 µl of RNase-Free Water to each tube. Mix well by repeated pipetting or vortexing at medium speed.
5. Repeat Steps 2 and 3.

6. Remove the 1.5-ml microcentrifuge tube from the magnetic stand. Add 65 µl of Magnetic Bead Resuspension Solution to each tube. Mix well by repeated pipetting or vortexing at medium speed.
7. Optional: Add 1 µl of RiboGuard RNase Inhibitor to each tube of resuspended Magnetic Beads, and mix briefly by vortexing.
8. Store the microcentrifuge tubes at room temperature until required in Part 3.C.

3.B. Treatment of the Total RNA Sample with Ribo-Zero rRNA Removal Solution

Required in Part 3.B

Component Name	Tube Label	Cap Color
Ribo-Zero Reaction Buffer	Ribo-Zero Reaction Buffer	Blue
Ribo-Zero rRNA Removal Solution (Human/Mouse/Rat)	rRNA Removal Solution-(H/M/R)	
RNase-Free Water	RNase-Free Water	Clear

Additionally required for each reaction (provided by user):
Magnetic stand or rack
0.2-ml or 0.5-ml microcentrifuge tube (RNase-free)

Important! *The maximum volume of the RNA sample and the volume of the Ribo-Zero rRNA Removal Solution used per reaction is dependent on the amount of total RNA in the sample (see Table 1).*

Table 1. Volumes of Ribo-Zero™ rRNA Removal Solution.

Amount of Input Total RNA	Maximum Volume of Total RNA That Can Be Added to Each Reaction	Volume of Ribo-Zero rRNA Removal Solution Used per Reaction
1-2.5 µg	28 µl	8 µl
2.5-5 µg	26 µl	10 µl

1. In a 0.2-ml or 0.5-ml RNase-free microcentrifuge tube, combine in the order given:

x	µl	RNase-Free Water
4	µl	Ribo-Zero Reaction Buffer
1-5	µg	Total RNA sample (see Table 1)
y	µl	Ribo-Zero rRNA Removal Solution (see Table 1)
40	µl	Total volume

2. Gently mix the reaction(s) by pipetting and incubate at 68°C for10 minutes. Store the remaining Ribo-Zero rRNA Removal Solution and Ribo-Zero Reaction Buffer at –70°C to –80°C.
3. Remove the reaction tube(s) and incubate each at room temperature for 5 minutes.

3.C. Magnetic Bead Reaction and rRNA Removal

Required in Part 3.C: 50°C water bath or heating block for 2.0-ml tubes.

1. Using a pipette, add the treated RNA from Part 3.B to the 1.5-ml microcentrifuge tube containing the washed Magnetic Beads and, without changing the pipet tip, immediately and thoroughly mix the contents of the tube by pipetting at least 10 times. Then, vortex the tube immediately at medium setting for 10 seconds and place at room temperature. Repeat this process for each sample.

⚠ ***Important!*** *Always add the treated RNA sample to the washed Magnetic Beads and immediately mix by pipetting. Never add the Magnetic Beads to the treated RNA sample. Immediate and thorough mixing prevents the beads from forming clumps that can significantly impact the efficiency of the rRNA removal.*

2. Incubate the 1.5 ml microcentrifuge tube at room temperature for 5 minutes.
3. Following incubation, mix the reactions by vortexing at medium speed for 5 seconds and then place at 50°C for 5 minutes in an appropriate water bath or heating block. Avoid any significant condensation during this incubation step.
4. After the 5-minute incubation at 50°C, remove the microcentrifuge tubes and immediately place them on a magnetic stand for at least 1 minute (until the solution appears clear).
5. Carefully remove each supernatant (85-90 µl) containing the RNA and transfer to a labeled 1.5-ml RNase-free microcentrifuge tube.

⚠ ***Important!*** *The supernatant contains rRNA-depleted RNA.*

Optional: *If a small amount of Magnetic Beads is still visible in the supernatant, place the collected supernatant onto the magnetic stand for 1 minute. Remove the supernatant containing the rRNA-depleted RNA and transfer to a new 1.5-ml RNase-free microcentrifuge tube.*

6. Place the supernatant (RNA solution) on ice and immediately proceed to Part 3.D. Alternatively, the supernatant may be stored at –70°C to –80°C before completing Part 3.D.

3.D. Purification of the rRNA-Depleted Sample

The rRNA-depleted samples can be purified by three methods: ethanol precipitation, AMPure beads, or spin columns. Ethanol precipitation and the modified RNeasy MinElute procedure provide optimal recovery of small RNAs that may be lost with other purification methods; however, ethanol precipitation can be challenging for inexperienced users. We also provide an alternative protocol using AMPure beads (provided by the user) for ease of automation but this will not quantitatively recover small RNAs.

3.D.1. Ethanol Precipitation of the rRNA-Depleted Sample

Component Name	Tube Label	Cap Color
RNase-Free Water	RNase-Free Water	Clear
Sodium Acetate (3 M)	Sodium Acetate	
Glycogen (10 mg/ml)	Glycogen	

Additionally required for each reaction (provided by user):
1.5-ml microcentrifuge tube (RNase-free)
Ice-cold 70% and 100% ethanol

1. Adjust the volume of each sample to 180 µl using RNase-Free Water.
2. Add 18 µl of 3 M Sodium Acetate to each tube.
3. Add 2 µl of Glycogen (10 mg/ml) to each tube and mix by *gentle* vortexing.
4. Add three volumes (600 µl) of ice-cold 100% ethanol to each tube and mix thoroughly by *gentle* vortexing.
5. Place the tubes at –20°C for at least 1 hour.
6. Centrifuge the tubes at >10,000 x g in a microcentrifuge for 30 minutes. Carefully remove and discard the supernatant.
7. Wash the pellet with ice-cold 70% ethanol and centrifuge at >10,000 x g for 5 minutes. Carefully remove and discard the supernatant.
8. Repeat Step 7 (above) one more time.
9. Centrifuge briefly to collect any residual supernatant. Carefully remove and discard the supernatant and allow the pellet to air dry at room temperature for 5 minutes.
10. Dissolve the pellet in the desired volume of RNase-Free Water or buffer. The rRNA-depleted RNA can be used immediately or stored at –70°C to –80°C.

3.D.2. Agencourt RNAClean XP Kit (Cat. No. A63987, Beckman Coulter)

Note: *A fresh 80% ethanol solution is required for Steps 5 and 7 below.*

1. Vortex the AMPure RNAClean XP Beads until they are well dispersed, then add as follows:

   Add 160 µl of the mixed AMPure XP Beads to each 1.5-ml microcentrifuge tube containing 85-90 µl of rRNA-depleted RNA from Part 3.C, Step 6. Mix thoroughly by gently pipetting the entire volume 10 times.
2. Incubate the tube(s) at room temperature for 15 minutes. During incubation, prepare 80% ethanol solution required for Steps 5 and 7.
3. Place the tube(s) on the magnetic stand at room temperature for at least 5 minutes (until the liquid appears clear).
4. Remove and discard the supernatant from each tube. Take care not to disturb the beads.
5. With the tube(s) still on the magnetic stand, add 200 µl of freshly prepared 80% ethanol to each tube, without disturbing the beads.
6. Incubate at room temperature for at least 30 seconds while still on the magnetic stand. Then remove and discard all of the supernatant from each tube. Take care not to disturb the beads.
7. Repeat Steps 5 and 6 (total of two 80% ethanol washes).
8. Allow the tubes to air dry on the magnetic stand at room temperature for 15 minutes.
9. Add a desired elution volume of RNase-Free water to each tube.

Note: *For rRNA-depleted RNA samples that will be used directly in a ScriptSeq™ v2 RNA-Seq Library Preparation reaction, we recommend eluting with 11 µl of RNase-Free Water in order to yield a final eluted volume of 9 µl.*

10. Thoroughly resuspend the beads by gently pipetting 10 times.
11. Incubate the tubes at room temperature for 2 minutes.
12. Place the tubes back onto the magnetic stand at room temperature for at least 5 minutes (until the liquid appears clear).
13. Transfer the clear supernatant from each tube to an appropriate collection tube, always leaving at least 1 µl of the supernatant behind to avoid carryover of magnetic particles. Store on ice for immediate use or store at –70°C or –80°C until required.

3.D.3. RNeasy MinElute Cleanup Kit (Cat. No. 74204, Qiagen)

Note: *RNA purification kits from other suppliers may also be used; however, performance may vary.*

1. Adjust the sample to a volume of 100 µl with RNase-Free Water. Add 350 µl of Buffer RLT, and mix well.
2. Add 550 µl of 96%-100% ethanol to the diluted RNA, and mix well by pipetting. Do not centrifuge.
3. Transfer half of the sample (~500 µl) to an RNeasy MinElute spin column placed in a 2-ml collection tube (supplied in the Qiagen kit). Close the lid gently, and centrifuge for 15 seconds at 8,000 x g (~10,000 rpm). Discard the flow-through. Reuse the collection tube for Step 5.
4. Transfer the remaining sample and repeat the centrifugation. Discard the flow-through and collection tube.
5. Place the RNeasy MinElute spin column in a new 2-ml collection tube (supplied in the Qiagen kit). Add 500 µl Buffer RPE to the spin column. Close the lid gently, and centrifuge for 15 seconds at 8,000 x g (~10,000 rpm) to wash the spin-column membrane. Discard the flow-through. Reuse the collection tube for Step 6.

   Note: *Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use.*
6. Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 minutes at 8,000 x g (~10,000 rpm) to wash the spin-column membrane. Discard the flow-through and collection tube.

   Note: *After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.*
7. Place the RNeasy MinElute spin column in a new 2-ml collection tube (supplied in the Qiagen kit).
8. Open the lid of the spin column, and centrifuge at full speed for 5 minutes. Discard the flow-through and collection tube.
9. To avoid damage to the spin-column lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (i.e., if the rotor rotates clockwise, orient the lids counterclockwise).

10. It is important to dry the spin-column membrane since residual ethanol may interfere with downstream applications. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.
11. Place the RNeasy MinElute spin column in a new 1.5-ml collection tube (supplied in the Qiagen kit). Add 14 µl of RNase-Free Water directly to the center of the spin-column membrane. Close the lid gently, and centrifuge for 1 minute at full speed to elute the RNA. Recovery is usually 10 µl.

    The eluted RNA can be used immediately or stored at –70°C to –80°C.

4. Quantifying the Yield and Assessing the Quality of the rRNA-Depleted Sample

The yield of rRNA-depleted RNA is dependent on the amount of input total RNA, the rRNA content of the sample, and the method used to purify the Ribo-Zero treated RNA in Part 3.D of the procedure.

Typically 3%-10% of the input RNA is recovered in the rRNA-depleted sample after ethanol precipitation.

If assessing the quality of the rRNA-depleted RNA using an Agilent 2100 Bioanalyzer, use the Agilent RNA6000 Pico Chip and load 2-4 ng of the rRNA-depleted RNA. The Agilent RNA Nano Chip does not provide sufficient sensitivity.

When purifying the rRNA-depleted RNA by ethanol precipitation, small RNAs such as miRNA and tRNA are recovered along with the mRNAs and large noncoding RNAs. Therefore, the presence of a high proportion of small RNA in the rRNA-depleted sample should not be interpreted as degradation of the RNA.

5. Related Products

Cat. #	Quantity
Ribo-Zero™ Magnetic Gold Kit (Human/Mouse/Rat)	
MRZG126	6 Reactions
MRZG12324	24 Reactions
Removes >99% of both cytoplasmic (nuclear-encoded) and mitochondrial rRNAs from human, mouse, or rat total RNA preparations.	
Ribo-Zero™ Magnetic Kit (Bacteria)	
MRZMB126	6 Reactions
MRZB12424	24 Reactions
Removes >99% of the rRNA from mixed populations of Gram-positive and Gram-negative bacterial RNA preparations.	
Ribo-Zero™ Magnetic Kit (Gram-Negative Bacteria)	
MRZGN126	6 Reactions
Removes >99% of the rRNA from Gram-negative bacteria total RNA preparations.	
Ribo-Zero™ Magnetic Kit (Gram-Positive Bacteria)	
MRZGP126	6 Reactions
Removes >99% of the rRNA from Gram-positive bacteria total RNA preparations.	
Ribo-Zero™ Magnetic Kit (Plant Leaf)	
MRZPL116	6 Reactions
Removes >99% of cytoplasmic and chloroplast rRNAs.	
Ribo-Zero™ Magnetic Kit (Plant Seed/Root)	
MRZSR116	6 Reactions
Removes >99% of cytoplasmic and chloroplast rRNAs.	
ScriptSeq™ v2 RNA-Seq Library Preparation Kit	
SSV21106	6 Reactions
SSV21124	24 Reactions

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