MGIEasy

rRNA Depletion Kit User Manual

Cat. No.: 1000005953 (32 RXN)

Kit Version: V1.1

Manual Version: A2

Revision History

Manual Version	Kit Version	Date	Description
A2	V1.1	Oct. 2019	◆ Add 16S rRNA in Introduction. ◆ Add $OD_{260/230}$ requirement in total RNA Quality Requirements. ◆ Add RNA cleanup beads volume in Table 11.
A1	V1.1	Oct. 2018	◆ Initial release.
A0	V1.0	-	◆ N/A

Note: Please download the latest version of the manual to use the corresponding kit.

Contents

Chapter 1 Product Description

1.1 Introduction

MGIEasy rRNA Depletion Kit depletes rRNA from human, mouse and rat total RNA (including cytoplasmic 5 S rRNA, 5.8 S rRNA, 18 S rRNA, 28 S rRNA, mitochodrial ribosomal RNA 12 S rRNA, 16 S rRNA and 45 S pre-ribosomal RNA). The resulting mRNA and other non-coding RNA are suitable for downstream RNA analysis applications. This kit is compatible with both intact and degraded RNA (e.g. FFPE RNA). The library prepared by using this kit with RNA Library Prep Kit (e.g. MGIEasy RNA Library Prep Kit or MGIEasy RNA Directional Library Prep Kit) is suitable for RNA quantitation, transcriptome or non-coding RNA research applications.

1.2 Application

This kit is compatible with total RNA samples from all human, mouse and rat.

1.3 Contents

MGIEasy rRNA Depletion Kit supports the preparation of 32 reactions (Cat. No.: 1000005953) and its components are listed in table 1:

Table 1 MGIEasy rRNA Depletion Kit (32 RXN) (Cat. No.: 1000005953)

Modules & Cat. No.	Components	Color Coded Screw Caps	Spec. & Quantity
MGIEasy rRNA Depletion Kit Cat. No.: 1000005953	Probe Mix	White	76.8 μL/tube × 1 tube
	Hybridization Buffer	White	192 μL/tube × 1 tube
	RNase H Buffer	White	115.2 μL/tube × 1 tube
	RNase H	White	76.8 μL/tube × 1 tube
	DNase I Buffer	White	576 μL/tube × 1 tube
	DNase I	White	192 μL/tube × 1 tube

1.4 Storage Conditions and Shelf Life

- Storage temperature: -25℃ to-18℃
- Production Date and Expiry Date: refer to the label
- Transport Conditions: transported in dry ice

* Please ensure that enough dry ice remains after transportation.

* Performance of products are guaranteed until the expiration date under appropriate transport, storage and usage conditions.

1.5 Equipment and Materials Required but not Provided

Before the experiment, prepare the following equipment and materials in Table 2. The “optional” material is determined by the actual requirement. For example, if DNase I digestion is used for eliminating contaminating DNA in RNA sample, the digested product needs to be quantified. Therefore, you need to prepare “Qubit™ 3.0 Fluorometer”. If rRNA-depleted RNA sample needs the band distribution detection, you need to prepare "Agilent 2100 Bioanalyzer" and reagents.

Table 2 Equipment and Materials Required but not Provided

Equipment	Vortex mixer
	Desktop Centrifuge
	Pipets
	Thermocycler（Bio-Rad Thermal Cycler with thermal gradient capability）
	Magnetic rack DynaMagTM-2（Thermo Fisher Scientific™，Cat. No. 12321D）or equivalent
	Qubit™ 3 Fluorometer（Thermo Fisher Scientific™, Cat. No. Q33216）(optional)
	Agilent 2100 Bioanalyzer （Agilent Technologies™, Cat. No. G2939AA）
Reagents	Nuclease free water, NF water（Ambion™, Cat. No. AM9937）or equivalent
	RNase Zap（Ambion™, Cat. No. AM9780）
	RNA cleanup beads（Agencourt RNAClean XP 40 mL Kit, Agencourt™, Cat. No. A63987）
	100% Ethanol (Analytical Grade)
	DNase I (NEB™, Cat. No. M0303S) (optional)
	Qubit™ RNA HS Assay Kit (Invitrogen™, Cat. No. Q32852) (optional)
	Agilent RNA 6000 Pico Kit (Agilent Technologies™, Cat. No. 5067-1513) (optional)
Consumables	RNase-free pipette tips
	1.5 mL RNase-free non-stick microcentrifuge tubes (Ambion™, Cat. No. AM12450)
	0.2 mL RNase-free PCR tubes (Axygen, Cat. No. PCR-02-C) or 96-well plate (Axygen™, Cat. No. PCR-96M2-HS-C)

1.6 Precautions and Warnings

- Instructions provided in this manual are intended for general use only, and it may require further adjustments to optimize performance. We recommend making adjustments while taking into account the experimental design, sample characteristics and other equipment for optimization.
- Wear masks and gloves before preparing RNA samples. Spray and wipe the pipette, test tube rack and bench with RNase Zap RNase Decontamination Solution.
- Retrieve the reagents from storage beforehand, and prepare them for use: For Enzymes, centrifuge briefly and place on ice for further use. For other modules, first defrost at room temperature and invert several times to mix properly. Finally, centrifuge briefly and place on ice for further use. If you can not consume the reagents in six times, please dispense the reagent into aliquots after the initial thaw and refreeze the aliquots in order to avoid excessive freeze-thaw cycles. However, if you aliquot, you may not have enough reagents for the full number of reactions over multiple use.
- When preparing mixtures and working solutions, we recommend pipetting up and down at least 10 times to mix thoroughly. Note that vigorous shaking may cause a decrease in yield.
- To prevent cross contamination, we recommend using filtered Pipette Tips. Use a new tip each time for pipetting different solutions.
- We recommend using Thermocyclers with Heated Lids for reactions. Preheat to reaction temperature before use. The temperature of Heated lid would be 105°C if there is no special illustration.
- To avoid contamination in the experiments, we recommend using designated pipettes and other equipments. Perform regular cleaning to ensure a sterile working environment (Use 0.5% Sodium Hypochlorite or 10% Bleach to clean working environment).
- If you have other questions, please contact MGI technical support: MGI-service@genomics.cn

Chapter 2 Sample Preparation

2.1 Compatible Sample Type and Input Requirements

This kit depletes rRNA from human, mouse and rat total RNA. It is compatible with FFPE sample (See Appendix B for instructions). For RNA sample with RIN (RNA Integrity Number) ≥7, the recommended amount of RNA input is 10 ng - 1 μg. For low-quality RNA sample (RIN< 7), the RNA input should not be less than 200 ng.

2.2 Total RNA Quality Requirements

- Use Agilent 2100 Bioanalyzer to perform quality for total RNA sample. RIN value should be ≥7. If RIN< 7, increase RNA input (no more than 1 μg) and the PCR cycles appropriately in the library construction. If RIN is N/A, the sample is unqualified for library construction.
- RNA purity: $OD_{260/280}$=1.8~2.0, $OD_{260/230}$≥2. The rRNA ratio will increase obviously when $OD_{260/230}$≤0.2. If $OD_{260/230}$<2, total RNA is recommended to be purified with RNA cleanup beads before Chapter 3. The purification method can refer to 3.4 RNA cleanup.
- Ensure no DNA contamination in RNA sample to maximize the effect of rRNA depletion. If DNA contamination is found in RNA sample (use agarose electrophoresis to detect), perform DNase I digestion to remove DNA. Please refer to Appendix A for instructions.
- If RNA sample is insufficient, you can try lower amount of input for depletion. However, lower amount of input might cause a decreased yield of PCR products and lower comparison rate in analysis result.

Chapter 3 Library Construction Protocol

This protocol is designed for rRNA depletion from 200 ng total RNA (UHRR, Universal Human Reference RNA) with RIN ≥7.

⚠ **Note: In the following procedures, do not vortex samples. Please mix solutions thoroughly by pipetting.**

3.1 RNA/Probe Hybridization

3.1.1 According to the concentration of total RNA, transfer appropriate RNA sample (recommended 200 ng total RNA) to 0.2 mL PCR tube, and add NF water to an 18 μL total volume.

⚠ **Warning: Please vortex Probe Mix 5-6 times, 3-5 s each, to mix thoroughly before use. When preparing reaction mixture, please add Probe Mix and Hybridization Buffer individually to RNA sample. Do not prepare Probe Mix and Hybridization Buffer mixture.**

3.1.2 Prepare RNA/Probe hybridization reaction mixture on ice (see Table 3):

Table 3 RNA/Probe Hybridization Reaction Mixture

Components	Volume
Total RNA	18 μL
Hybridization Buffer	5 μL
Probe Mix	2 μL
Total	25 μL

3.1.3 Pipette the mixture at least 10 times to mix. Place samples in a thermocycler, set up the reaction conditions following Table 4 and start the reaction:

Table 4 The Reaction Conditions of RNA/Probe Hybridization

Temperature	Time
Heated lid	On
95°C	2 min
95°C-22°C	0.1°C /s
22°C	5 min

3.1.4 The reaction takes approximately 20 min. After 22°C for 5 min, immediately remove the tube and place it on ice for 2 min. Spin down the samples in a tabletop centrifuge and proceed immediately to the next step.

3.2 RNase H Digestion

3.2.1 Prepare RNase H reaction mixture on ice (see Table 5):

Table 5 RNase H Digestion Reaction Mixture

Components	Volume
Product in Step 3.1.4	25 μL
RNase H	2 μL
RNase H Buffer	3 μL
Total	30 μL

3.2.2 Pipette the mixture at least 10 times to mix. Place the tube in the Thermocycler, set up the reaction conditions following Table 6 and start the reaction:

Table 6 The Reaction Conditions of RNase H Digestion

Temperature	Time
Heated lid (45℃)	On
37℃	30 min
4℃	∞

⚠ **Note: If a heated lid cannot be set to 45℃, please set the temperature to the one closest to 45℃.**

3.2.3 Centrifuge briefly to collect the samples to the bottom of the tube and proceed immediately to the next step.

3.3 DNase I Digestion

3.3.1 Prepare DNase I digestion reaction mixture on ice (see Table 7):

Table 7 DNase I Digestion Reaction Mixture

Components	Volume
Product in Step 3.2.3	30 μL
DNase I	5 μL
DNase I Buffer	15 μL
Total	50 μL

3.3.2 Pipette the mixture at least 10 times to mix. Place the tube in the Thermocycler, set up the reaction conditions following Table 8 and start the reaction:

Table 8 The Reaction Conditions of DNase I Digestion

Temperature	Time
Heated lid (45℃)	on
37℃	30 min
4℃	∞

3.3.3 Centrifuge briefly to collect the samples to the bottom of the tube.

3.3.4 Transfer all products to a new 1.5 mL RNase-free non-stick tube.

3.4 RNA Cleanup

⚠ **Note: Please read Appendix C carefully before you begin. Please use RNase-free non-stick tube in the RNA cleanup.**

3.4.1 Remove RNA Cleanup beads from 4℃ storage and place it at room temperature for 30 min beforehand. Vortex to mix thoroughly before use.

3.4.2 Transfer 75 μL resuspended RNA Cleanup beads to the RNA sample from Step 3.3.4.

3.4.3 Gently pipette at least 10 times to mix thoroughly. The color of the liquid should appear homogeneous. Be careful to expel all of the liquid out of the tip during the last mix.

3.4.4 Incubate at room temperature for 5 minutes.

3.4.5 Centrifuge briefly. Place the tube on the magnetic rack for 2-5 minutes until liquid clears. Carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the RNA.

3.4.6 Keep the tube on the magnetic rack and add 200 μL freshly prepared 80% ethanol to the tube Incubate at room temperature for 30 seconds and carefully remove and discard the supernatant.

3.4.7 Repeat Step 3.4.6 once for a total of two washes, remove all liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid to the bottom, place back on the magnet and then remove liquid using a small volume pipette tip.

3.4.8 Keep the tube on the magnetic rack with the lid open and allow beads to air dry until no wetness (reflectiveness) is observed. Do not over-dry the beads.

3.4.9 Remove the tube from the magnetic rack and add appropriate amount of NF water to elute the RNA. Pipette at least 10 times to mix thoroughly.

⚠ **Note: In Step 3.4.8 Elute the RNA sample from the beads, please elute the RNA by adding appropriate volume of NF water according to the following application. For example, if you use MGIEasy RNA**

Library Prep Kit or MGIEasy RNA Directional Library Prep Kit for library construction, you need to add 12 μL NF water to elute RNA and transfer 10 μL of supernatant to proceed to RNA fragmentation reaction.

3.4.10 Incubate the tube at room temperature for 5 minutes.

3.4.11 Centrifuge briefly. Place the tube on the magnetic rack for 2-5 minutes until liquid is clears. Transfer the supernatant to a new RNase-free PCR tube.

3.4.12 Cleanup sample can be placed on ice for NGS library construction or other application. The product can be stored at -20°C overnight or at -80°C for no more than one week (We recommend you immediately proceed to the next reaction).

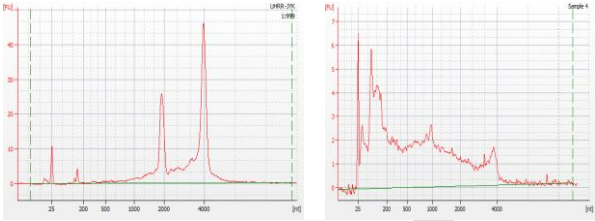
3.5 Quality Control of rRNA Depletion Product

After the cleanup of rRNA depleted RNA sample, please perform the quality control following the instructions:

⚠ **Note: This quality control is optional. If rRNA depleted RNA sample is proceeded to the NGS library construction, it is recommended to skip this step.**

3.5.1 Take 1 μL of the cleanup sample for quality control using the Agilent RNA 6000 Pico chip (it is not provided, see "Equipment and Material Required but not Provided". Please refer to the Agilent RNA 6000 Pico chip manual for the instructions) and Agilent 2100 Bioanalyzer. In Figure 1A, two significant 18 S and 28 S rRNA peaks are presented in the Agilent 2100 analysis result of the UHRR sample without rRNA depletion. In Figure 1B, no 18 S and 28 S rRNA peaks are showed in the Agilent 2100 analysis result of the rRNA depleted UHRR sample. This result demonstrates the effectiveness of rRNA depletion using MGIEasy rRNA Depletion Kit.

⚠ **Note: Agilent RNA Nano chip does not meet the sensitivity requirement for the RNA quality control.**



A B

Figure 1 Agilent RNA 6000 Pico assay result of purified rRNA depleted RNA

A. The Agilent 2100 analysis result of the UHRR sample without rRNA depletion.

B. The Agilent 2100 analysis result of the rRNA depleted UHRR sample.

3.5.2 According to the assay result, after removal of rRNA, the total amount of remaining RNA should not exceed 8% of the total input. For example, if the input amount of total RNA is 200 ng, after removal of rRNA, the total amount of remaining RNA should not exceed 16 ng.

Appendix

Appendix A DNase I Digestion of RNA Sample

If there is DNA contamination in RNA sample, you need to perform DNase I digestion (materials are not provided, see "Equipment and Materials Required but not Prepared"). DNase I digestion cause a certain loss of RNA sample, the amount of total RNA used in this step needs to be increased by 20% to 30% than the expected input (the amount of RNA input for rRNA depletion required in this kit). For example, for rRNA depletion, if the required input amount of RNA is 200 ng, the total RNA input should be 250-286 ng for the DNase I digestion. The protocol is shown below:

a) Transfer an appropriate amount of RNA sample to a RNase free 0.2 mL PCR tube and add NF water to the final volume of 42.5 μL. Prepare the DNase I digestion reaction mixture on ice (see Table 9):

Table 9 DNase I Digestion Reaction Mixture

Components	Volume
total RNA	42.5 μL
DNase I	2.5 μL
10× DNase I Buffer	5 μL
Total	50 μL

b) Gently pipette the mixture and place the tube in the Thermocycler. Set up the reaction conditions following Table 10 and start the reaction:

Table 10 The Reaction Conditions of DNase I Digestion

Temperature	Time
Heated lid (45°C)	on
37°C	20 min
4°C	∞

c) When the temperature reaches 4°C, centrifuge the PCR tube briefly and transfer the mixture to a new 1.5 mL non-stick tube. Use 90 μL RNAcleanup beads for cleanup. The cleanup procedures are shown below:

⚠ **Note: Please read Appendix C carefully before you begin. Please use non-stick tubes in this RNA cleanup.**

1) Remove RNAClean XP beads from 4°C storage and place it at room temperature for 30 min beforehand. Vortex to mix thoroughly before use.

2) Transfer 90 μL RNAClean XP beads to DNase I digestion product in Step 3. Gently Pipette at least 10 times to mix thoroughly. Be careful to expel all of the liquid out of the tip into the tube during the last mix.

3) Incubate the tube at room temperature for 5 minutes.

4) Centrifuge briefly. Place the tube on the magnetic rack for 2-5 minutes until liquid clears. Carefully remove the supernatant with a pipette.

5) Keep the tube on the magnetic rack and add 200 µL freshly prepared 80% ethanol with NF water to the tube without disturbing the beads. Incubate for 30 seconds and carefully remove and discard the supernatant.

6) Repeat step 5 for a total of two washes, remove all liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid to the bottom, place back on the magnet and then remove liquid using a small volume pipette tip.

7) Keep the tube on the magnetic rack with the lid open and allow beads to air dry until no wetness (reflectiveness) is observed. Do not over-dry the beads.

8) Remove the tube from the magnetic rack and add appropriate amount of NF water to elute the RNA. Pipette at least 10 times to mix thoroughly.

⚠ **Note: If the purified RNA sample needs to be quantitated, elute the RNA by adding 21 µL of NF water, transfer 19 µL of supernatant to a new RNase free PCR tube and take 1µL of product for quantitation using "Qubit™ RNA HS Assay Kit" (See "Equipment and Materials Required but not Provided"). If the quantitation is not performed, elute the RNA by adding 20 µL of NF water and transfer 18 µL of supernatant to a new RNase free PCR tube for RNA/Probe hybridization.**

9) Incubate the tube at room temperature for 5 minutes.

10) Centrifuge briefly. Place the non-stick tube on the magnetic rack for 2-5 minutes until liquid is clear. Carefully transfer the supernatant to a new RNase free PCR tube and proceed to the RNA/Probe hybridization.

Appendix B FFPE Sample Preparation

This kit is also applicable to low-quality total RNA samples such as FFPE. However, due to the large difference between the quality of different FFPE samples, it is not guaranteed that libraries can be prepared from all FFPE samples. The following instructions take the NGS library construction using the "MGIEasy RNA Library Prep kit" as an example and list the problems that you need to pay attention to during the NGS library construction from FFPE samples with different qualities.

B-1 Quality Evaluation of FFPE Sample

The RIN value is the most common parameter for the evaluation of RNA quality. However, the RIN value cannot accurately assess the quality of the degraded FFPE sample. Particularly, in the NGS library construction, the RIN value of the FFPE sample is not always proportional to the overall success rate of library construction. Therefore, DV_{200} is also used for assessing the success rate of library construction from FFPE samples. The DV_{200} indicates the proportion of RNA fragments larger than 200 nt in the sample. For severely degraded FFPE samples, the DV_{200} value is a reliable indicator for the sample quality.

The calculation of DV_{200}

Take the analysis result from Agilent 2100 Bioanalyzer as an example for the DV_{200} calculation. Detailed calculation is shown in Figure 2:

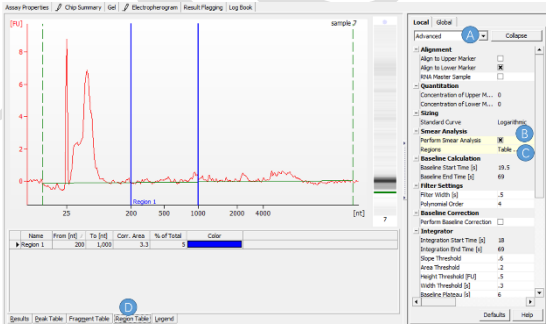


Figure 2 The Calculation of DV_{200}

A: In the Agilent 2100 Bioanalyzer result figure, choose *Advanced* under *Local*

B: Check the *Perform Smear Analysis* option under *Smear Analysis*

C: Double-click on *Table* to enter the range of fragments to be calculated. The figure shows the example of *From* 200 bp *To* 1000 bp.

D: Obtain the proportion of selected fragment shown as *% of Total* in the *Region Table*

If you need to determine the DV_{200} parameters of the FFPE sample, perform the Agilent 2100 Bioanalyzer analysis (using the RNA analysis chip) on the FFPE sample, and calculate DV_{200} according to the above method. For detailed information, please see *DV200 determination for FFPE RNA samples.* (https://www.agilent.com/en/promotions/dv200-determination)

B-2 Recommended Input Amount of FFPE Sample

For FFPE samples, we recommend using different total RNA inputs for samples with different DV_{200} value in the rRNA depletion. In addition, if the rRNA depleted RNA sample is used for NGS library construction, use difference conditions for fragmentation reaction and different number of PCR cycles in PCR amplification. See Table 11 for detailed conditions.

Table 11 The Recommended Conditions for Library Construction from FFPE Sample

DV_{200} Value	Recommended amount of total RNA input	RNAClean XP beads	Conditions of fragmentation	PCR cycles
> 70%	200 ng	75 μL	94℃，8 min	14
50-70%	200-400 ng	100 μL	94℃，8 min	16
30-50%	500 ng	100 μL	94℃，6 min	16
< 30%	0.5-1 μg (with a risk of failure of NGS library construction)	100 μL	No fragmentation	16

Note:

a. “Conditions of fragmentation” in the above table refers to the conditions of fragmentation in “RNA fragmentation” step in “MGIEasy RNA Library Prep Kit” or “MGIEasy RNA Directional Library Prep Kit”.

b. “PCR cycles” in the above table refers to the PCR cycles in “PCR amplification” step in “MGIEasy RNA Library Prep Kit” or “MGIEasy RNA Directional Library Prep Kit”.

FFPE samples from different tissues may have different performances. This table is for reference only. The specific library construction strategy needs to be adjusted according to different RNA sample.

Appendix C Magnetic Beads Cleanup

The Agencourt RNAClean XP 40 mL Kit is used to purify RNA samples in this kit. If other beads are used, need to test and determine the specific purification conditions.

Before you use

- To ensure capture efficiency of the Magnetic Beads, remove beads from 4°C refrigerator storage, and let it stand at RT for 30 min beforehand. Vortex and mix thoroughly before use.
- Vortex or pipette up and down to ensure that the beads are thoroughly mixed every time they are used.

Operation Notes

- If the sample volume decreases due to evaporation during incubation, add TE buffer to designated volume before using beads to purify.
- During the magnetic separation stage, please allow the solution to become completely clear before removing the supernatant. This process takes approximately 2-3 min. Take into consideration the varying magnetic strength of your specific Separation Plate / Rack, and allow enough time for the solution to turn completely clear.
- Avoid touching the beads while pipetting. 2-3 μL of fluids can be left in the tube to avoid contact. In case of contact between the beads and pipette tip, expel all solution and beads to the tube and restart the separation process.
- Use freshly prepared 80% Ethanol (at room temperature) to wash the beads. The non-stick tube should remain on the magnetic rack when washing. Do not shake or disturb the beads.
- After the 2nd washing of beads with Ethanol, try to remove all liquid within the tube. You may centrifuge briefly to collect any remaining liquid to the bottom, separate beads magnetically, and remove remaining liquid by using a small volume pipette.
- After washing twice with Ethanol, air dry beads at room temperature. Insufficient drying (observed by a reflective surface) will cause Anhydrous Ethanol to deposit, affecting subsequent reactions. Over-drying (observed by cracking of pellet) may cause a reduction in yield. Drying takes approximately 5-10 min depending on your specific lab environment. Observe closely, until the pellet appears sufficiently dry with a matt appearance, then continue to the elution process with TE Buffer.
- Avoid disturbing beads while removing the Supernatant. Contamination from the beads may affect subsequent reactions. Therefore, the total volume of TE Buffer and beads can be 2 μL more than the volume of the supernatant.

- Pay attention when opening / closing the lids of 1.5 mL tubes on the rack. Strong vibrations may cause sample loss through spilling liquid or bead. Secure the tubes well before opening with the lids.

■ Contact Information

Company: MGI Tech Co., Ltd
Address: 2/F, Building 11, Beishan Industrial Zone, Yantian District, Shenzhen, CHINA, 518083
Website: http://en.mgitech.cn
Email: MGI-service@genomics.cn
Service Hotline: (+86) 4000-966-988