Ribo-MagOff rRNA Depletion Kit (Human/Mouse/Rat)

N420

Nanjing Vazyme Biotech Co.,Ltd.
Tel: +86 25-83772625
Email: info.biotech@vazyme.com
Web: www.vazyme.com
Loc: Red Maple Hi-tech Industry Park, Nanjing, PRC
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Instruction for Use

Version 22.1

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01/Product Description

Ribo-MagOff rRNA Depletion Kit (Human/Mouse/Rat) is designed for removing ribosomal RNA (rRNA) (including cytoplasmic 28S, 18S, 5.8S, 5S rRNA, and mitochondrial 16S, 12S rRNA) from total RNA of humans, mice, and rats while retaining mRNA and other non-coding RNA. The kit is applicable to both intact and partially degraded RNA samples (e.g. FFPE samples), and the resulting ribosomal-depleted RNA can be used for the analysis of mRNA and non-coding RNA (such as lncRNA).

02/Components

	Component	N420-01 (12 rxns)	N420-02 (24 rxns)
BOX 1	Reaction Buffer	12 μl	24 μl
	Removal Solution	12 μl	24 μl
	Nuclease-free ddH_2O	1 ml	2 × 1 ml
BOX 2	Magnetic Beads	2 × 720 μl	4 × 720 μl
	Magnetic Bead Resuspension Solution	180 μl	360 μl

03/Storage

BOX 1, Store at -30 ~ -15℃ and transport at ≤0℃.
BOX 2, Store at 2 ~ 8℃. Adjust the shipping method according to the destination.

04/Applications

Ribo-MagOff rRNA Depletion Kit (Human/Mouse/Rat) is suitable for removing rRNA (including cytoplasmic 28S, 18S, 5.8S, 5S rRNA, and mitochondrial 16S, 12S rRNA) from total RNA samples with an initial template input of 0.01 - 1 μg while retaining mRNA and other non-coding RNA. The kit is applicable for both intact and partially degraded RNA samples, and the resulting products are suitable for RNA library preparation and other experimental uses. The mRNA level in total RNA varies significantly across different samples, and the initial input of total RNA can be appropriately adjusted according to the downstream applications. The resulting products can be used for transcriptome library preparation with VAHTS Universal V8 RNA-seq Library Prep Kit for Illumina (Vazyme #NR605).

05/Self-prepared Materials

◇ RNA quality control: Agilent RNA 6000 Pico Kit (Agilent #5067-1513).

◇ Magnetic beads for RNA purification: VAHTS RNA Clean Beads (Vazyme #N412).

◇ Other materials: 80% ethanol (freshly prepared with Nuclease-free ddH_2O), Nuclease-free ddH_2O; Nuclease-free PCR tubes; Agilent 2100 Bioanalyzer or other equivalent products, PCR instrument, magnetic stand, etc.

06/Notes

For research use only. Not for use in diagnostic procedures.

06-1/About Storage

1. Store BOX 1 at -30 ~ -15℃ and keep it on ice during use. Store BOX 2 at 2 ~ 8℃. Store the reagents under the specified conditions immediately after use.
2. To avoid the degradation of Removal Solution due to repeated freeze-thaw cycles or long-time use, it is recommended to store the remaining reagents in small aliquots after the first use.

06-2/About RNA Sample Preparation

1. To ensure rRNA depletion efficiency, RNA samples should be free of salt ions (such as Mg^{2+} or guanidine salts) or organic compounds (such as phenol or ethanol), or the samples need to be purified again.
2. To avoid DNA contamination, RNA samples can be treated with DNase I to remove DNA.
3. Do not leave the RNA on ice for a long time after dilution to 8 μl with Nuclease-free ddH_2O to avoid RNA degradation.
4. If the initial RNA volume is >8 μl due to the low concentration, the RNA can be concentrated by lyophilization, ethanol precipitation, column-based or magnetic bead-based purification (using VAHTS RNA Clean Beads, Vazyme #N412), or other methods.
5. For RNA-seq, it is recommended to start with over 100 ng of input total RNA to increase library complexity.

06-3/About Magnetic Beads

1. The magnetic beads should be equilibrated to room temperature after being taken out from the 2 ~ 8℃ environment. Otherwise, the gripping efficiency of the magnetic bead will be affected.
2. The magnetic beads should be thoroughly mixed by vortexing every time before pipetting.
3. The supernatant should be carefully removed after the beads are completely adsorbed (the supernatant becomes clear) while the tube is kept on the magnetic stand. Avoid disturbing the beads.
4. When purifying the ribosomal-depleted RNA, make sure to use 80% ethanol (freshly prepared with Nuclease-free ddH_2O) to rinse the magnetic beads, otherwise, RNA loss may occur.

06-4/About Operation

1. It is recommended to use filter pipette tips; change tips when pipetting different samples.
2. Always wear gloves; change gloves after contact with the equipment outside the RNase-free area or entering other work areas.
3. All reagents must be capped immediately after use to avoid contamination.

07/Mechanism & Workflow

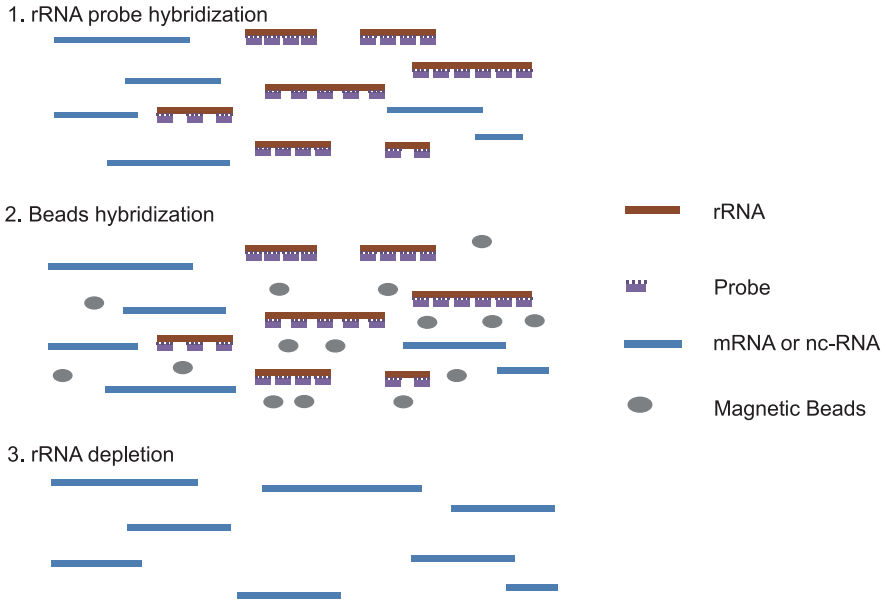

Fig 1. Schematic Diagram of rRNA Depletion

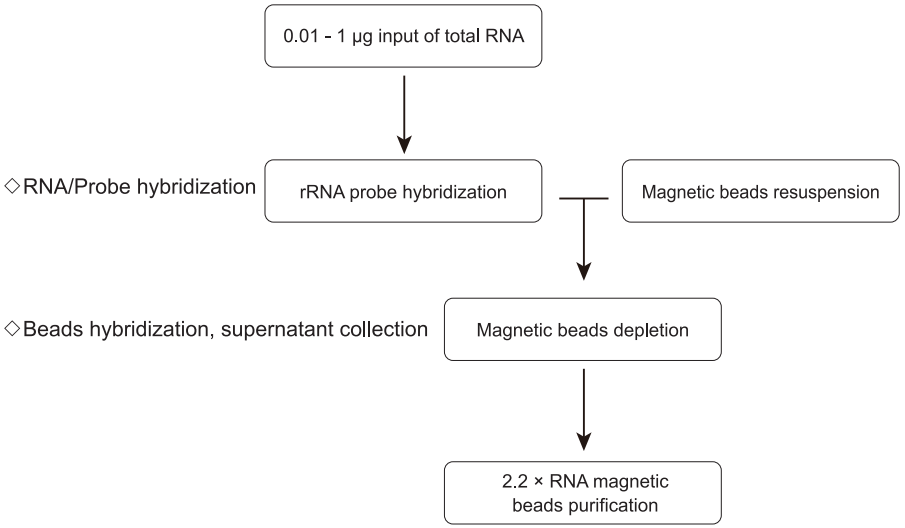

Fig 2. Workflow of rRNA Depletion

08/Experiment Process

08-1/Magnetic Beads Preparation

1. Mix the magnetic beads by vortexing, pipette 120 μl into a 0.2 ml Nuclease-free centrifuge tube. Place the tube on the magnetic stand. After the solution becomes clear (about 5 min), carefully remove the supernatant.
2. Remove the centrifuge tube from the magnetic stand, add 120 μl of Nuclease-free ddH_2O, and thoroughly resuspend the beads by pipetting up and down 10 times. Hold the centrifuge tube on the magnetic stand. After the solution becomes clear (about 5 min), carefully remove the supernatant.
3. Repeat Step 2 once.
4. Remove the centrifuge tube from the magnetic stand, add 15 μl of Magnetic Bead Resuspension Solution, and mix by vortexing to resuspend the beads. Store the beads at room temperature for later use.

08-2/Probe Hybridization

A. For high-integrity RNA samples

1. Prepare the total RNA sample: Dilute 0.01 - 1 μg of total RNA with Nuclease-free ddH_2O in a 0.2 ml Nuclease-free centrifuge tube to 8 μl and keep the tube on the ice for subsequent use.
2. Prepare the following reaction mix in an Nuclease-free centrifuge tube.

Components	Volume
Reaction Buffer	1 μl
Removal Solution	1 μl
Total RNA	8 μl
Total	10 μl

Mix the solution thoroughly by gently pipetting up and down 10 times. Collect the solution to the bottom of the tube by brief centrifugation.

3. Load the sample into the PCR instrument to allow probe hybridization.

Temperature	Time
68℃	10 min

Immediately remove the tube upon completion of the reaction, collect the solution to the bottom of the tube by brief centrifugation, and allow it to stand at room temperature for 5 min.

B. For FFPE samples or severely degraded RNA samples

1. Prepare the total RNA sample: Dilute 0.01 - 1 μg of total RNA with Nuclease-free ddH_2O in a 0.2 ml Nuclease-free centrifuge tube to 8 μl and keep the tube on the ice for subsequent use.
2. Prepare the following reaction mix in a Nuclease-free centrifuge tube.

Components	Volume
Reaction Buffer	1 μl
Removal Solution	1 μl
Total RNA	8 μl
Total	10 μl

Mix the solution thoroughly by gently pipetting up and down 10 times. Collect the solution to the bottom of the tube by brief centrifugation.

3. Load the sample into the PCR instrument to allow probe hybridization.

Temperature	Time
95℃	2 min
95 ~ 37℃	0.1℃/sec
37℃	5 min

Immediately remove the tube upon completion of the reaction, collect the solution to the bottom of the tube by brief centrifugation, and proceed to the next step immediately.

08-3/Magnetic Bead Capture

1. Prepare the following reaction mix.

Components	Volume
Magnetic Bead Resuspension Solution	15 μl
Products from the previous step	10 μl
Total	25 μl

2. Immediately mix the solution thoroughly by gently pipetting up and down 10 times.
3. Incubate the sample at room temperature for 5 min to allow hybridization with magnetic beads.
4. Load the sample into the PCR instrument and run the following program.

Temperature	Time
50℃	5 min

5. Place the Nuclease-free centrifuge tube on the magnetic stand upon completion of the reaction. After the solution becomes clear (about 5 min), carefully transfer 23 μl of the supernatant into a new 0.2 ml Nuclease-free centrifuge tube and place it on ice for later use.

08-4/RNA Purification

1. Mix the VAHTS RNA Clean Beads thoroughly by vortexing. Pipette 50.6 μl (2.2 ×) of the beads into the RNA sample obtained from the previous step. Mix the solution thoroughly by pipetting up and down 10 times.
2. Incubate the sample on ice for 15 min to allow the RNA to bind to the magnetic beads.
3. Place the sample on the magnetic stand. After the solution becomes clear (about 5 min), carefully remove the supernatant.

4. Keep the sample on the magnetic stand. Add 200 µl of 80% ethanol (freshly prepared with Nuclease-free ddH_2O) to rinse the beads. Incubate the sample at room temperature for 30 sec and carefully remove the supernatant.

5. Repeat Step 4 once.

6. Keep the sample on the magnetic stand. Uncap the tube and air-dry the beads at room temperature for 5 - 10 min.

▲ Do not resuspend the magnetic beads when adding 80% ethanol.

▲ Use a 10 µl pipette to remove the residual supernatant.

a. If the purified product is used for reverse transcription, remove the sample from the magnetic stand, add 20 µl of Nuclease-free ddH_2O and mix thoroughly by pipetting up and down 6 times, and allow to stand at room temperature for 2 min. Keep the sample on the magnetic stand. After the solution becomes clear (about 5 min), carefully transfer 18 µl of the supernatant into a new Nuclease-free PCR tube. Store the tube at -80℃ for later use.

b. If the purified product is used for transcriptome library preparation, e.g. with VAHTS Universal V8 RNA-seq Library Prep Kit for Illumina (Vazyme #NR605), remove the sample from the magnetic stand, add 18.5 µl of 1× Frag/Prime Buffer, mix thoroughly by pipetting up and down 6 times, and allow to stand at room temperature for 2 min. Keep the sample on the magnetic stand. After the solution becomes clear (about 5 min), carefully transfer 16 µl of the supernatant into a new Nuclease-free PCR tube, and prepare the library immediately.

09/FAQ & Troubleshooting

◇ How can the purified products be stored?

The purified products are easily degraded due to low concentration. Proceed to downstream experiments as soon as possible, otherwise store at -80 ~ -65℃.

◇ What if the purified product is intended for library preparation but is eluted with Nuclease-free ddH_2O?

When using VAHTS Universal V8 RNA-seq Library Prep Kit for Illumina (Vazyme #NR605), If conditions permit, add an equal volume of 2 × Frag/Prime Buffer . The reaction system is amplified then until the purification step in which the system is to be restored. You can also use VAHTS RNA Clean Beads (Vazyme #N412) to purify again and elute with 1×Frag/Prime Buffer in the final step.

◇ If the starting library concentration is too low, what can be done to find the cause and solve the problem?

The yield of RNA after rRNA depletion depends on the quality of the initial RNA, the content of rRNA in the sample and the purification method used. The concentration of the library constructed with high-quality RNA samples as templates can meet the requirements of sequencing. If qualified RNA samples cannot be obtained, you can try to use the following methods to make up:

① Initial amount: increase the initial amount of sample, the upper limit is 1 µg;

② Make repetitions and merge them after the purification step;

③ No size selection: Although the RNA fragments are small under the fragmentation condition of 94℃ for 8 min, the distribution will be concentrated and the uniformity will be better.

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