Ribo-clean rRNA Depletion Kit Mega (Bacteria)

RN417

Vazyme

Instruction for Use

Version 24.1

Contents

01/Product Description 02
02/Components 02
03/Storage 02
04/Applications 02
05/Self-prepared Materials 03
06/Notes 03
- 06-1/About Storage 03
- 06-2/About RNA Sample Preparation 03
- 06-3/About RNA Magnetic Beads 04
- 06-4/About Operation 04

07/Mechanism & Workflow 05
08/Experiment Process 05
- 08-1/RNA Hybridization with Probe 05
- 08-2/RNase H Digestion 06
- 08-3/DNase I Digestion 07
- 08-4/Ribosomal-depleted RNA Purification 07

09/FAQ & Troubleshooting 08

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

Ribo-clean rRNA Depletion Kit Mega (Bacteria) is designed to remove ribosomal RNA (rRNA) from total RNA of Gram-positive and Gram-negative bacteria, compatible with the initial total RNA input of 0.01 - 1 μg. The total RNA sample undergoes steps such as rRNA-probe hybridization, RNase H digestion and DNase I digestion, ultimately removing rRNA (including 5S, 16S, 23S rRNA) from the total RNA and retaining mRNA and other non-coding RNA. This kit is applicable for both intact and partially degraded RNA samples, and the resulting RNA can be used for mRNA and non-coding RNA (e.g., lncRNA) analysis. The optimized reaction system improves the depletion efficiency and species compatibility.

02/Components

Components	RN417-01 (6 rxns)	RN417-02 (12 rxns)	RN417-03 (24 rxns)
rRNA Probe Mega (Bacteria)	18 μl	36 μl	72 μl
Probe Buffer	18 μl	36 μl	72 μl
RNase H Buffer 2	18 μl	36 μl	72 μl
RNase H Mix	12 μl	24 μl	48 μl
DNase I Buffer	174 μl	348 μl	696 μl
DNase I	6 μl	12 μl	24 μl
Nuclease-free ddH_2O	1 ml	1 ml	1 ml

▲ The colors marked in the table represent the cap colors of each components tube.

03/Storage

Store at -30 ~ -15°C and ship at ≤0°C.

04/Applications

Ribo-clean rRNA Depletion Kit Mega (Bacteria) is applicable for rRNA depletion (including 5S, 16S, 23S rRNA) from total RNA of Gram-positive and Gram-negative bacteria with an initial input of 0.01 - 1 μg. It is compatible with partially degraded RNA samples, and the resulting product is applicable for RNA library preparation and other experiments. The mRNA content in total RNA from different samples varies greatly, and the initial template input of total RNA can be appropriately adjusted according to downstream applications. The resulting product can be used for library preparation with VAHTS Universal V8 RNA-seq Library Prep Kit for Illumina (Vazyme #NR605), VAHTS Universal V10 RNA-seq Library Prep Kit for Illumina (Vazyme #NR606), or VAHTS Universal V10 RNA-seq Library Prep Kit (Premixed Version) (Vazyme #NR616).

05/Self-prepared Materials

◇ RNA evaluation

Agilent RNA 6000 Pico Kit (Agilent #5067-1513).

◇ RNA purification

VAHTS RNA Clean Beads (Vazyme #N412).

◇ Library Preparation

VAHTS Universal V8 RNA-seq Library Prep Kit for Illumina (Vazyme #NR605),

VAHTS Universal V10 RNA-seq Library Prep Kit for Illumina (Vazyme #NR606),

VAHTS Universal V10 RNA-seq Library Prep Kit (Premixed Version) (Vazyme #NR616).

◇ Other materials

80% ethanol (Nuclease-free ddH_2O freshly prepared), Nuclease-free ddH_2O; Nuclease-free PCR tubes; Agilent 2100 Bioanalyzer or other equivalent products, PCR instrument, magnetic rack (Vazyme #CM101), etc.

06/Notes

06-1/About Storage

1. The kit contains a variety of enzymes, which must be stored at -30 ~ -15°C. When in use, they should be placed on ice and promptly stored under the specified conditions after use, otherwise, the enzyme activity may be reduced.
2. To avoid repeated freezing and thawing, it is recommended to aliquot the remaining reagent into small portions for storage after the first use.

06-2/About RNA Sample Preparation

1. In order to ensure depletion efficiency, RNA samples should not contain salt ions (e.g., Mg^{2+} or guanidine salts) or organic substances (e.g., phenol or ethanol), otherwise, re-purification should be performed.
2. To avoid DNA contamination, RNA samples can be treated with DNase I to remove DNA.
3. Dilute RNA with Nuclease-free ddH_2O to 9 μl and do not leave it on ice for a long time to avoid RNA degradation.
4. If the RNA concentration is too low, resulting in an initial volume >9 μl, methods such as lyophilization, ethanol precipitate, column recovery or magnetic beads purification (VAHTS RNA Clean Beads, Vazyme #N412) can be used for concentration.
5. For samples used in RNA-Seq, it is recommended that the total RNA input be higher than 100 ng to increase library complexity.

06-3/About RNA Magnetic Beads

1. Magnetic beads should be equilibrated to room temperature after being taken out from 2 ~ 8°C, otherwise, it will affect the recovery efficiency. Before each pipetting of the magnetic beads, they should be vortexed and mixed thoroughly.
2. The supernatant should be carefully removed after the beads are completely adsorbed (the supernatant becomes clear) while the tube is kept on the magnetic rack. Avoid disturbing the beads.
3. When purifying Ribosomal-depleted RNA, be sure to use 80% ethanol (Nuclease-free ddH_2O freshly prepared) to rinse magnetic beads, otherwise it may cause RNA loss, directly leading to library preparation failure. After the second 80% ethanol rinse of the magnetic beads, try to discard the supernatant as much as possible to minimize impurity residues.
4. Ensure that the magnetic beads are fully dried before elution (the surface changes from shiny brown to matte brown) to avoid ethanol residue affecting subsequent experiments, but over drying (cracking) will result in RNA loss.

06-4/About Operation

1. It is recommended to use filter pipette tips and change tips when pipetting different samples.
2. Be sure to wear gloves during operation, and change gloves after touching equipment or other work areas outside the RNase-free space.
3. All reagents must be covered immediately after use to avoid contamination.
4. Briefly centrifuge enzyme components before use to avoid adhesion to the tube wall and cap, causing loss.

07/Mechanism & Workflow

Fig 1. Schematic Diagram of rRNA Depletion

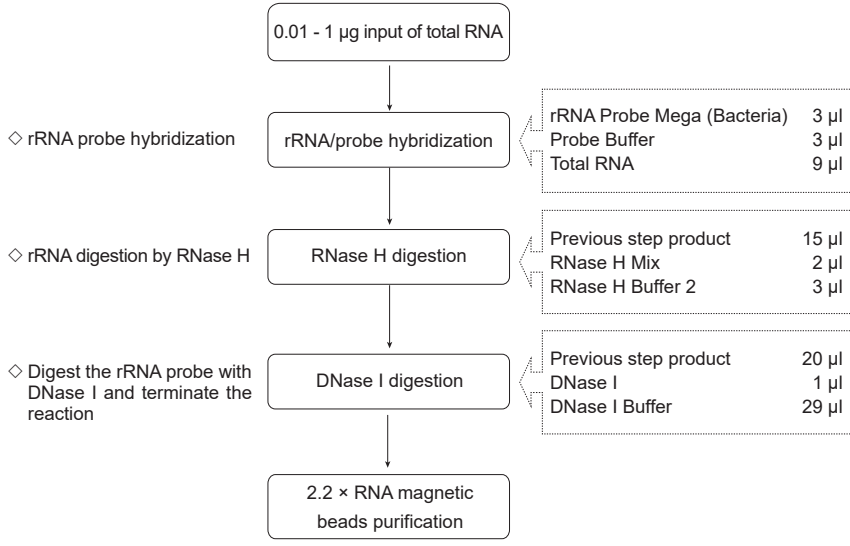

Fig 2. Workflow of rRNA Depletion

08/Experiment Process

08-1/RNA Hybridization with Probe

1. Dilute 0.01 - 1 μg of total RNA with Nuclease-free ddH_2O in the Nuclease-free PCR tube to 9 μl, place on ice for later use.

   ▲ Take out the components needed for the next step from -30 ~ -15°C in advance and place them on ice for later use.

2. Prepare the following reaction mix in the Nuclease-free PCR tube:

Components	Volume
rRNA Probe Mega (Bacteria)	3 µl
Probe Buffer	3 µl
Total RNA	9 µl
Total	15 µl

3. Use a pipette to gently pipette up and down 10 times to mix thoroughly, and briefly centrifuge to collect at the bottom of the tube.

▲ If multiple samples are processed simultaneously, the rRNA Probe Mega (Bacteria) and Probe Buffer mixture can be pre-prepared in a suitably sized centrifuge tube and then aliquoted into each PCR tube. It is recommended to prepare 1.1 times the actual number of reactions to compensate for losses.

4. Place the PCR tubes in the PCR instrument and perform the following program:

Temperature	Time
95°C	2 min
95 ~ 37°C	0.1°C/sec
37°C	5 min
4°C	Hold

5. Immediately take out after the reaction is completed. Briefly centrifuge to collect the sample at the bottom of the tube, and place it on ice for the next step.

08-2/RNase H Digestion

1. Prepare the following reaction mix on ice:

Components	Volume
RNase H Buffer 2	3 µl
RNase H Mix	2 µl
Previous step product	15 µl
Total	20 µl

2. Use a pipette to gently pipette up and down 10 times to mix thoroughly, and briefly centrifuge to collect at the bottom of the tube.

3. Place the PCR tubes in the PCR instrument and perform the following program:

Temperature	Time
50°C	15 min
4°C	Hold

4. Immediately take out after the reaction is completed. Briefly centrifuge to collect the sample at the bottom of the tube, and place it on ice for the next step.

08-3/DNase I Digestion

1. Prepare the following reaction mix on ice:

Components	Volume
DNase I Buffer	29 µl
DNase I	1 µl
Previous step product	20 µl
Total	50 µl

2. Use a pipette to gently pipette up and down 10 times to mix thoroughly, and briefly centrifuge to collect at the bottom of the tube.

3. Place the PCR tubes in the PCR instrument and perform the following program:

Temperature	Time
37°C	10 min
4°C	Hold

4. Immediately take out after the reaction is completed. Briefly centrifuge to collect the sample at the bottom of the tube, and place it on ice for the next step.

08-4/Ribosomal-depleted RNA Purification

1. Mix VAHTS RNA Clean Beads (Vazyme #N412) thoroughly by vortexing. Add 110 µl (2.2 ×) to the RNA sample from the previous step, and pipette up and down 10 times to mix thoroughly.

2. Incubate on ice for 15 min, allowing RNA to bind to the magnetic beads.

3. Keep the tube on the magnetic rack until the supernatant is clear (~ 5 min), carefully discard the supernatant.

4. Keep the tube on the magnetic rack and wash the beads with 200 µl 80% ethanol (Nuclease-free ddH_2O freshly prepared) without disturbing the beads. Incubate at room temperature for 30 sec, carefully discard the supernatant.

5. Repeat step 4 once.

6. Keep the sample always on the magnetic rack, open the lid and air-dry the beads at room temperature for 5 - 10 min.
   ▲ Do not disturb the magnetic beads when adding 80% ethanol.
   ▲ Use a 10 µl pipette to remove the residual supernatant.
   ▲ Avoid over drying (cracking) of the magnetic beads to prevent reduced recovery efficiency.

A: If the purified product is used for reverse transcription reaction, remove the sample from the magnetic rack and add 20 µl Nuclease-free ddH_2O. Pipette up and down 6 times to mix thoroughly, keep at room temperature for 2 min. Place the sample on the magnetic rack until the supernatant is clear (~ 5 min). Carefully transfer 18 µl of the supernatant into a new Nuclease-free PCR tube, and store at -80°C for later use.

B: If the purified product is used for transcriptome library preparation, such as VAHTS Universal V10 RNA-seq Library Prep Kit for Illumina (Vazyme #NR606), remove the sample from the magnetic rack and add 18 μl Frag/Prime Buffer 2. Use a pipette to mix thoroughly by pipetting up and down 6 times, keep at room temperature for 2 min. Place the sample on the magnetic rack until the supernatant is clear (~ 5 min). Carefully transfer 16 μl of the supernatant into a new Nuclease-free PCR tube and immediately proceed with library preparation.

09/FAQ & Troubleshooting

◇ How to store the purified product?

The purified product is prone to degradation due to its low concentration. Proceed to downstream experiments as soon as possible, otherwise store at -80 ~ -65℃.

◇ If the purified product is used for library preparation with Nuclease-free ddH_2O elution, how to operate?

When using the VAHTS Universal V10 RNA-seq Library Prep Kit for Illumina (Vazyme #NR606), add an equal volume of VAHTS 2 × Frag/Prime Buffer V2 (Vazyme #N405) if conditions permit. Then scale up the reaction system until the purification step, and restore the system. Alternatively, VAHTS RNA Clean Beads (Vazyme #N412) can be used for purification again, and finally elute with Frag/Prime Buffer.

◇ If the purified product is used for library preparation, how should the fragmentation conditions and number of amplification cycles be selected?

The yield of RNA after rRNA depletion depends on the quality of the starting RNA, the rRNA content and the purification method used. The concentration of the library prepared from high-quality RNA samples can meet the requirements for sequencing. If qualified RNA samples cannot be extracted, the following methods can be tried to compensate:

1. Initial input: Increase the initial input of the sample, up to 1 μg.
2. Make several duplicate samples and combine them together after the purification step.
3. Do not use the size selection scheme: Under the condition of 94°C for 8 min, although the fragments are relatively small, the distribution will be concentrated and the uniformity is also good.

◇ If rRNA depletion is required for multiple species, can multiple probes be used simultaneously?

Multiple probes can be used simultaneously. The amount of probe used can be adjusted according to the recommended usage in the manual, while reducing the amount of RNA input.

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