Ribo-off rRNA Depletion Kit V2 (Bacteria)

N417

Instruction for Use

Version 22.1

Vazyme

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01/Product Description

The Ribo-off rRNA Depletion Kit V2 (Bacteria) is designed to remove ribosomal RNA (rRNA) from the total RNA of gram-positive and gram-negative bacteria. This kit is compatible with an initial template input of 0.01 - 1 μg. The total RNA sample undergoes probe hybridization, RNase H digestion, DNase I digestion, and other steps for the rRNAs (including 16S and 23S rRNA) to be depleted while retaining mRNA and other non-coding RNA. This kit is applicable to both intact and partially degraded RNA samples, and the resulting RNA can be used for the analysis of mRNA and non-coding RNA (such as LncRNA). The optimized reaction system enhances the depletion effect and shortens the workflow time.

02/Components

	Components	N417-01 (12 rxns)	N417-02 (24 rxns)
■ (purple)	rRNA Probe V2 (Bacteria)	24 μl	48 μl
■ (purple)	Probe Buffer	36 μl	72 μl
■ (orange)	RNase H Buffer 2	36 μl	72 μl
■ (orange)	RNase H Mix	24 μl	48 μl
■ (green)	DNase I Buffer	348 μl	696 μl
■ (green)	DNase I	12 μl	24 μl
□ (white)	Nuclease-free ddH_2O	1 ml	1 ml

▲The colors of tube caps for each kit component are indicated.

03/Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

04/Applications

The Ribo-off rRNA Depletion Kit V2 (Bacteria) is suitable for rRNA (including 16S and 23S rRNA) depletion from the total RNA of gram-positive and gram-negative bacteria with an initial template input of 0.01 - 1 μg while retaining mRNA and other non-coding RNA. The kit is compatible with partially degraded RNA samples, and the resulting products are suitable for RNA library preparation and other experiments. The mRNA level in total RNA varies significantly across different samples, and the initial template input of total RNA can be adjusted appropriately according to the downstream applications. The resulting products can be used for 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).

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

◇ Library preparation kits: VAHTS Universal V8 RNA-seq Library Prep Kit for Illumina (Vazyme #NR605).

◇ Other materials: 80% ethanol (freshly prepared with Nuclease-free ddH_2O), Nuclease-free ddH_2O; Nuclease-free PCR tubes, low-adsorption EP tubes (Eppendorf #022431021); 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. The kit contains a variety of enzymes and must be stored at -30 ~ -15℃. It should be kept on ice during use and stored under the specified conditions immediately after use; otherwise, the enzyme activity may be reduced.
2. To avoid the decrease in enzyme activity 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 trace DNA.
3. Do not leave the RNA on ice for a long time after dilution to 10 μl with Nuclease-free ddH_2O to avoid RNA degradation.
4. If the initial RNA volume is >10 μl due to the low concentration, the RNA can be concentrated by lyophilization, ethanol precipitation, column-based or magnetic bead-based purification (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 to ensure optimal capture efficiency.

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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 Ribosomal-depleted RNAs, make sure to use 80% ethanol (freshly prepared with Nuclease-free ddH_2O) to rinse the magnetic beads; otherwise, the RNA may be degraded, leading to library construction failure.
5. After rinsing the magnetic beads twice with 80% ethanol, do not leave any supernatant behind to minimize residual impurities.
6. The beads should be dry enough before elution (the surface changes from glossy brown to frosted brown) to prevent residual ethanol from affecting subsequent reactions; however, over-drying the beads (leading to surface cracks) may lead to a loss of RNA sample.

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.
4. The enzyme components should be briefly centrifuged before use to avoid adhesion to the tube wall and cap and resulting loss.

07/Mechanism & Workflow

1. rRNA probe hybridization

2. RNase H digestion

3. DNase I digestion

rRNA

mRNA or nc-RNA

rRNA Probe

Fig 1. Schematic Diagram of rRNA Depletion

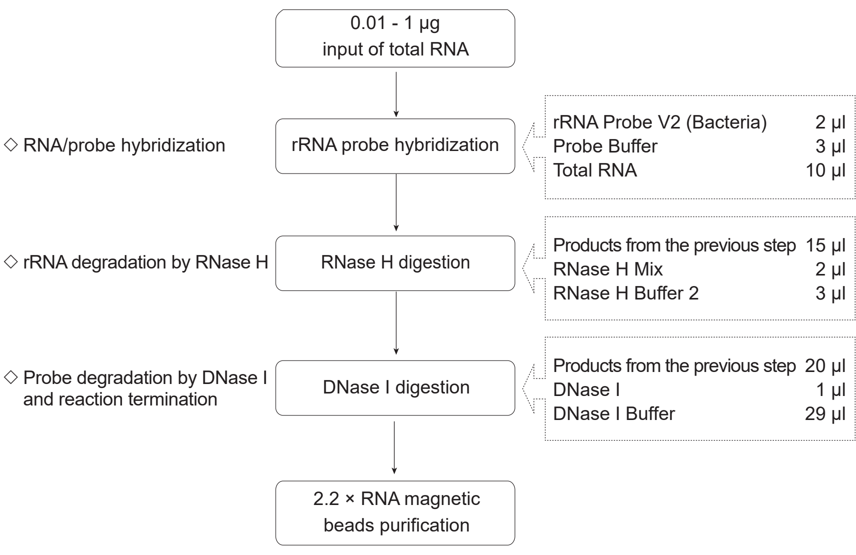

Fig 2. Workflow of rRNA Depletion

08/Experiment Process

08-1/Probe Hybridization

1. Dilute 0.01 - 1 μg of total RNA with Nuclease-free ddH_2O in an Nuclease-free PCR tube to 10 μl and keep the tube on ice for later use.

   ▲ The reagents needed for the next step can be taken out from the -30 ~ -15℃ storage in advance and placed on ice for later use.

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

Components	Volume
rRNA Probe V2 (Bacteria)	2 μl
Probe Buffer	3 μl
Total RNA	10 μl
Total	15 μl

Mix the solution thoroughly by gently pipetting up and down 10 times.

▲ If multiple samples are processed simultaneously, a mixture of rRNA Probe V2 (Bacteria) and Probe Buffer can be prepared in advance in a centrifuge tube of a suitable size and then aliquoted into each PCR tube. It is recommended to prepare the mixture at 1.1 times the needed volume for the actual reaction to compensate for the loss.

3. Collect the sample to the bottom of the tube by instantaneous centrifugation and load the sample into the PCR instrument. Run the following program, which takes approximately 10 - 15 min.

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

4. Remove the sample immediately after the reaction. Collect the sample to the bottom of the tube by instantaneous centrifugation, place the tube on ice, and proceed to 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
Products from the previous step	15 μl
Total	20 μl

Mix the solution thoroughly by gently pipetting up and down 10 times.

2. Load the sample into the PCR instrument and run the following program.

Temperature	Time
50℃	15 min
4℃	Hold

3. Collect the sample to the bottom of the tube by instantaneous centrifugation, place the tube on ice, and proceed to the next step immediately.

08-3/DNase I Digestion

1. Prepare the following reaction mix on ice.

Components	Volume
DNase I Buffer	29 μl
DNase I	1 μl
Products from the previous step	20 μl
Total	50 μl

Mix the solution thoroughly by gently pipetting up and down 10 times.

2. Load the sample into the PCR instrument and run the following program.

Temperature	Time
37℃	10 min
4℃	Hold

3. Collect the sample to the bottom of the tube by instantaneous centrifugation, place the tube on ice, and proceed to the next step immediately.

08-4/Ribosomal-depleted RNA Purification

1. Mix the VAHTS RNA Clean Beads (Vazyme #N412) thoroughly by vortexing. Pipette 110 μl (2.2 ×) of the beads into the RNA sample 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 disturb the magnetic beads when adding 80% ethanol.

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

▲ Avoid over-drying the magnetic beads (leading to surface cracks), which may result in low RNA recovery.

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 for 5 min. After the solution becomes clear, carefully transfer 18 μl of the supernatant into a new Nuclease-free PCR tube. Store the tube at -85 ~ -65℃ 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 for 5 min. After the solution becomes clear, 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-seqLibrary 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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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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www.vazyme.com