Ribo-clean rRNA Depletion Kit Mega (AA)

RN413

Vazyme

Instruction for Use

Version 25.1

Contents

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

01/Product Description

Ribo-clean rRNA Depletion Kit Mega (AA) (Aquatic Animals) is designed for rRNA depletion with total RNA from aquatic species, such as *Ostrea*, *Litopenaeus vannamei*, *Hexagrammos otakii*, and *Acipenser brevirostrum*. The kit is applicable to 0.01 - 1 μg total RNA, and rRNA (including 5S, 5.8S, 18S, 28S rRNA, 12S, and 16S rRNA) can be depleted through probe hybridization, RNase H digestion, and DNase I digestion. The kit is suitable for intact or partially degraded RNA samples, and the optimized reaction system improves depletion efficiency and species compatibility.

02/Target Species

Cat. No.	Application	Target Species*
RN413	Aquatic Animals	*Ostrea*/*Mytilus edulis*/*Litopenaeus vannamei*/*Macrobrachium rosenbergii*/*Hexagrammos otakii*/*Acipenser ruthenus*/*Cynops*/*Pelodiscus sinensis*/*Corallium rubrum*, etc.

* Species coverage can be found at *http://cloud.vazyme.com:83/application-tool*.

03/Components

Components	RN413-01 (6 rxns)	RN413-02 (12 rxns)	RN413-03 (24 rxns)
rRNA Probe Mega (AA)	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

04/Storage

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

05/Applications

Ribo-clean rRNA Depletion Kit Mega (AA) (Aquatic Animals) is suitable for the depletion of rRNA (including 5S, 5.8S, 18S, 28S rRNA, 12S, and 16S rRNA) with 0.01 - 1 μg total RNA from aquatic species, such as *Ostrea*, *Litopenaeus vannamei*, *Hexagrammos otakii*, and *Acipenser brevirostrum*. The kit is compatible with partially degraded RNA samples, and the depleted product is applicable to RNA library preparation and other experiments. The amount of mRNA varies significantly across different samples, and the initial total RNA inputs can be adjusted according to downstream applications. For RNA 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), and VAHTS Universal V10 RNA-seq Library Prep Kit (Premixed Version) (Vazyme #NR616) are recommended.

06/Self-prepared Materials

◇ RNA Evaluation

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

◇ RNA Purification

VAHTS RNA Clean Beads (Vazyme #N412).

◇ Library Preparation Kit

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 (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 rack (Vazyme #CM101), etc.

07/Notes

07-1/About Storage

1. The kit contains multiple enzymes and must be stored at -30 ~ -15°C. It should be kept on ice during use and returned to the specified storage conditions promptly after use; otherwise, enzyme activity may be reduced.
2. To avoid repeated freeze-thaw cycles, please aliquot and store the remaining reagent after first use.

07-2/About RNA Sample Preparation

1. To ensure depletion efficiency, RNA samples should be free of ionic salts (e.g., Mg^{2+} or guanidine salts) or organic substances (e.g., phenol or ethanol), or another purification is required.
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 avoid prolonged exposure on ice to prevent RNA degradation.
4. If a low RNA concentration results in an initial volume >9 μl, the RNA can be concentrated by lyophilization, ethanol precipitation, column purification or magnetic bead purification (VAHTS RNA Clean Beads, Vazyme #N412).
5. For RNA-Seq applications, the total RNA input >100 ng is recommended to increase library complexity.

07-3/About RNA Magnetic Beads

1. Equilibrate magnetic beads to room temperature and mix well before use, or the recovery efficiency may be affected.
2. Transfer the supernatant when the solution becomes clear and do not disturb the beads.
3. Use 80% ethanol (freshly prepared with Nuclease-free ddH_2O) to rinse the magnetic beads for ribosomal-depleted RNA purification; otherwise, it may cause RNA loss and library preparation failure. Please discard as much of the supernatant as possible to minimize impurity residues after second rinse.
4. Before elution, ensure that the magnetic beads are thoroughly dry (with the surface changing from shiny brown to matte brown) to prevent ethanol residue from affecting downstream experiments. However, it is important to avoid over-drying as it can reduce the RNA recovery efficiency.

07-4/About Operation

1. It is recommended to use pipette tips with filters and change the pipette tips for different samples.
2. Be sure to wear gloves during operation. Please change gloves after touching equipment or other work areas outside the RNase-free zones.
3. All reagents must be capped immediately after use to avoid contamination.
4. Briefly centrifuge enzyme components before use to avoid adhesion to the tube walls and caps.

08/Mechanism & Workflow

Fig 1. Schematic Diagram of rRNA Depletion

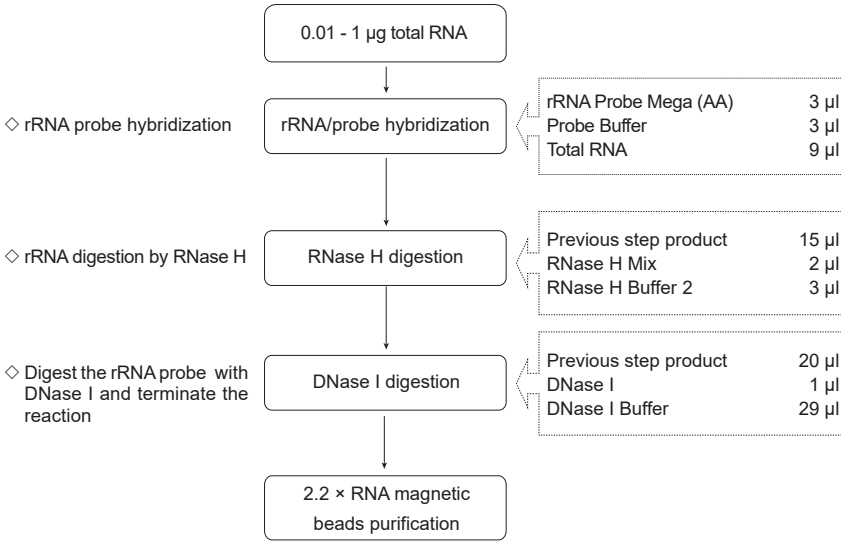

Fig 2. Workflow of rRNA Depletion

09/Experiment Process

09-1/Probe Hybridization with RNA

1. Dilute 0.01 - 1 μg total RNA with Nuclease-free ddH_2O to a final volume of 9 μl in a Nuclease-free PCR tube, and keep it on ice for later use.

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

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

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

   ▲ When multiple samples are processed simultaneously, the rRNA Probe Mega and Probe Buffer can be pre-mixed, and then aliquoted into each PCR tube. It is recommended to prepare 1.1 times the actual number of reactions to compensate for losses.

3. Mix well by pipetting 10 times, and briefly centrifuge to collect the reaction mix at the bottom of the tube.

4. Place the PCR tube 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

▲ Please note that 95 ~ 37°C is a gradient annealing program.

5. Remove the tube immediately when the reaction is completed. Briefly centrifuge to collect the reaction mix at the bottom of the tube, and place it on ice for the next step.

09-2/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. Mix well by pipetting 10 times, and briefly centrifuge to collect the reaction mix at the bottom of the tube.

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

Temperature	Time
50°C	15 min
4°C	Hold

4. Briefly centrifuge to collect the reaction mix at the bottom of the tube, and place it on ice for the next step.

09-3/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. Mix well by pipetting 10 times, and briefly centrifuge to collect the reaction mix at the bottom of the tube.

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

Temperature	Time
37°C	10 min
4°C	Hold

4. Briefly centrifuge to collect the reaction mix at the bottom of the tube, and place it on ice for the next step.

09-4/Ribosomal-depleted RNA Purification

1. Mix VAHTS RNA Clean Beads (Vazyme #N412) well by vortexing. Add 110 μl (2.2 ×) to the RNA sample from the previous step, and mix well by pipetting 10 times.
2. Incubate the tube on ice for 15 min to allow RNA to bind to the magnetic beads.
3. Place the tube on the magnetic rack, and discard the supernatant when the solution becomes clear (about 5 min).
4. Keep the tube on the magnetic rack, and wash the beads with 200 μl of 80% ethanol (freshly prepared with Nuclease-free ddH_2O). Incubate at room temperature for 30 sec, and then carefully discard the supernatant.
5. Repeat step 4 once.
6. Keep the tube on the magnetic rack, and air-dry the beads for 5 - 10 min.
   ▲ Do not disturb the magnetic beads when adding 80% ethanol.
   ▲ Use a 10 μl pipette to discard the residual ethanol.
   ▲ Avoid over-drying the magnetic beads, which may reduce recovery efficiency.
7. RNA elution:

Option A - Reverse transcription application:

Remove the tube from the magnetic rack, add 20 μl of Nuclease-free ddH_2O, and mix well. Incubate at room temperature for 2 min. Place the tube on the magnetic rack, and carefully transfer 18 μl of the supernatant to a new Nuclease-free PCR tube when the solution becomes clear (about 5 min). Store at -85 ~ -65°C for later use.

Option B - RNA library preparation application:

Remove the tube from the magnetic rack, add 18 μl of Frag/Prime Buffer 2 from VAHTS Universal V10 RNA-seq Library Prep Kit (Premixed Version) (Vazyme #NR616), and mix well. Incubate at room temperature for 2 min. Place the tube on the magnetic rack, and carefully transfer 16 μl of the supernatant to a new Nuclease-free PCR tube when the solution becomes clear (about 5 min).

Place the tube in a PCR instrument and perform fragmentation according to the following program: 85°C for 6 min, then hold at 4°C. Proceed to the subsequent steps of library preparation.

▲ For other fragmentation conditions, refer to Appendix/Table 1 in the manual of Vazyme #NR616.

10/FAQ & Troubleshooting

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

Multiple probes can be used simultaneously for rRNA depletion across various species. Follow the recommended amount of probe in the instruction manual and reduce the volume of RNA input to maintain a total volume of 15 μl.

Take multi-species rRNA depletion (Vazyme #RN413 + RN417) for example:

Components	Volume
rRNA Probe Mega (AA)	3 μl
rRNA Probe Mega (Bacteria)	3 μl
Probe Buffer	3 μl
Total RNA	6 μl
Total	15 μl

▲ Only the reaction system in **09-1/Probe Hybridization with RNA** is changed, while all other operations remain unchanged.

▲ If the initial amount of RNA is insufficient due to reduced volume, the RNA can be concentrated by VAHTS RNA Clean Beads (Vazyme #N412).

◇ How to know the species coverage and compatible products?

Please refer to *http://cloud.vazyme.com:83/application-tool* to get the species coverage. The species coverage is not the same as the depletion efficiency and is for reference only.

◇ How to store the purified product?

The purified product is prone to degradation due to low concentration. Proceed to the downstream experiments as soon as possible, or store at -85 ~ -65°C.

◇ If the purified product is used for library preparation, but it is eluted with Nuclease-free ddH_2O, what's the operation?

Purify the RNA again with VAHTS RNA Clean Beads (Vazyme #N412), and finally elute with Frag/Prime Buffer 2 from Vazyme #NR616.

◇ If the library concentration is too low, how to improve it?

Library concentration is related to total RNA quality including the integrity and input amounts. If it is not possible to extract qualified RNA samples, the following compensatory methods may be taken:

1. Increase total RNA inputs: up to 1 μg.
2. Prepare several duplicate samples and merge them after purification.

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