

Ribo-off rRNA depletion kit (Plant)

N409



Instruction for Use
Version 24.1

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

01/Product Description

Ribo-off rRNA depletion kit (Plant) is designed to deplete ribosomal RNA (rRNA) from the total RNA of plant roots, seeds, and leaves. The kit is applicable to 1 - 5 µg of total RNA, and rRNA (including 5S, 18S, 25S rRNA; mt5S, mt18S, mt26S rRNA; cp5S, cp4.5S, cp16S, cp23S 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/Components

Components	N409-01 (12 rxns)	N409-02 (24 rxns)
 rRNA Probe (Plant)	24 µl	48 µl
 Probe Buffer	36 µl	72 µl
 RNase H Buffer	48 µl	96 µl
 RNase H	12 µl	24 µl
 DNase I Buffer	348 µl	696 µl
 DNase I	12 µl	24 µl
☐ Nuclease-free ddH ₂ O	1 ml	2 × 1 ml

▲ The colors in the table correspond to the cap color of each component.

03/Storage

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

04/Applications

Ribo-off rRNA depletion kit (Plant) is suitable for rRNA depletion with 1 - 5 µg total RNA of plant roots, seeds, and leaves. The kit is compatible with partially degraded RNA sample, 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) is recommended.

05/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).
- ◇ Other Materials
80% ethanol (freshly prepared with Nuclease-free ddH₂O), Nuclease-free ddH₂O; Nuclease-free PCR tubes; Agilent 2100 Bioanalyzer or other equivalent products, PCR instrument, magnetic rack (Vazyme #CM101), etc.

06/Notes

06-1/About Application and Storage

1. The kit contains multiple enzymes, which must be stored at -30 ~ -15°C. It should be placed on the ice during use and stored as required after use, or the enzyme activity may be reduced.
2. To avoid repeated freeze-thaw cycles, please aliquot and store the remaining reagent after first use.

06-2/About RNA Sample Preparation

1. Dilute RNA with Nuclease-free ddH₂O to 10 µl or 11 µl and avoid prolonged exposure on ice to prevent RNA degradation.
2. If a low RNA concentration results in an initial volume >10 µl, the RNA can be concentrated by lyophilization, ethanol precipitation, column purification or magnetic bead purification (VAHTS RNA Clean Beads, Vazyme #N412).

06-3/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 ddH₂O) 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.

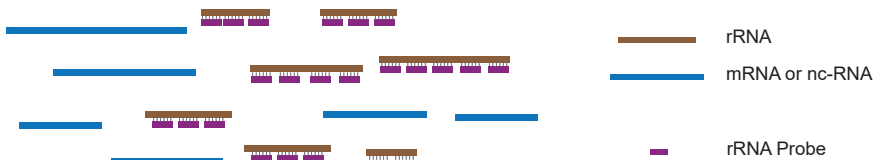
- 4. Before elution, ensure that the magnetic beads are thoroughly dry (with the surface changing from shiny brown to matte brown) to prevent ethanol residue from affecting downstream experiments. However, it is important to avoid over-drying as it can reduce the RNA recovery efficiency.

06-4/About Operation

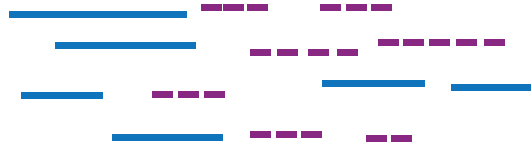
- 1. It is recommended to use pipette tips with filters and change the pipette tips for different samples.
- 2. Be sure to wear gloves during operation. Please change gloves after touching equipment or other work areas outside the RNase-free zones.
- 3. All reagents must be capped immediately after use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

07/Mechanism & Workflow

1. rRNA probe hybridization



2. RNase H digestion



3. DNase I digestion



Fig 1. Schematic Diagram of rRNA Depletion

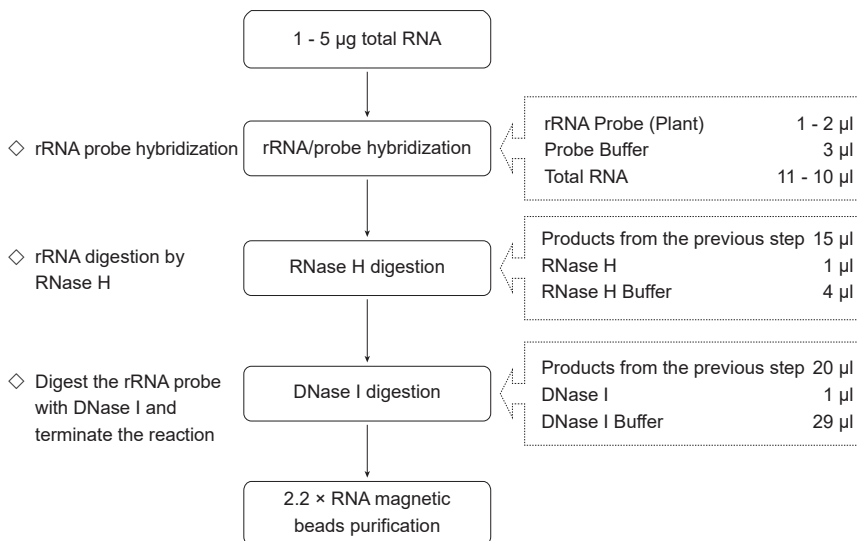


Fig 2. Workflow of rRNA Depletion

08/Experiment Process

08-1/Probe Hybridization with RNA

1. Dilute 1 - 5 µg total RNA with Nuclease-free ddH₂O to a final volume of 10 µl or 11 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	1 - 2.5 µg Initial Input	2.5 - 5 µg Initial Input
rRNA Probe (Plant)	1 µl	2 µl
Probe Buffer	3 µl	3 µl
Total RNA	11 µl	10 µl
Total	15 µl	15 µl

▲ When multiple samples are processed simultaneously, rRNA Probe (Plant) 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:

Procedure	Temperature	Time
Heated lid On, 105°C		
RNA denaturation	95°C	2 min
Probe hybridization	95 ~ 22°C	0.1°C/sec
Incubation	22°C	5 min
Hold	4°C	

▲ Please note that 95 ~ 22°C is a gradient annealing program.

- 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.

08-2/RNase H Digestion

- Prepare the following reaction mix on ice:

Components	Volume	
RNase H Buffer	4 µl	■
RNase H	1 µl	■
Products from the previous step	15 µl	
Total	20 µl	

▲ When multiple samples are processed simultaneously, RNase H Buffer and RNase H 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.

- Mix well by pipetting 10 times, and briefly centrifuge to collect the reaction mix at the bottom of the tube.
- Place the tube in the PCR instrument, and perform the following program:

Procedure	Temperature	Time
Heated lid On, 105°C		
RNase H digestion	37°C	30 min
Hold	4°C	

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

08-3/DNase I Digestion

- 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	

▲ When multiple samples are processed simultaneously, DNase I Buffer and DNase I 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.

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:

Procedure	Temperature	Time
Heated lid On, 105°C		
DNase I digestion	37°C	30 min
Hold	4°C	

4. Briefly centrifuge to collect the reaction mix 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) well by vortexing. Add 110 μ l (2.2 $\times$) 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 μ l of 80% ethanol (freshly prepared with Nuclease-free ddH₂O). 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 μ l 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 μ l of Nuclease-free ddH₂O, and mix well. Incubate at room temperature for 2 min. Place the tube on the magnetic rack, and carefully transfer 18 μ l 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:

1. Prepare Frag/Prime Buffer (1 ×) in a Nuclease-free tube:

Components	Volume
Nuclease-free ddH ₂ O	10 µl
2 × Frag/Prime Buffer	10 µl
Total	20 µl

▲ 2 × Frag/Prime Buffer is provided in VAHTS Universal V8 RNA-seq Library Prep Kit for Illumina (Vazyme #NR605).

2. Remove the tube from the magnetic rack, add 18 µl of prepared Frag/Prime Buffer (1 ×), 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), and immediately proceed to library preparation.

09/FAQ & Troubleshooting

◇ 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 ddH₂O, what's the operation?

Purify the RNA again with VAHTS RNA Clean Beads (Vazyme #N412), and finally elute with prepared Frag/Prime Buffer (1 ×).

◇ 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 5 µg.
- ② Prepare several duplicate samples and merge them after purification.



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