Ribo-off rRNA depletion kit (Plant)

N409

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
Follow Us

Instruction for Use

Version 22.1

Contents

01/Product Description

The Ribo-off rRNA Depletion Kit (Plant) is designed to remove ribosomal RNA (rRNA) from the total RNA of plant roots, seeds, and leaves. This kit is suitable for removing rRNA from plant total RNA with an initial template amount of 1 - 5 μg. The total RNA sample undergoes probe hybridization, RNase H digestion, DNase I digestion, and other steps for the rRNAs (including 5S, 18S, and 25S rRNA) to be depleted while retaining mRNA and other non-coding RNA, which can be used for the analysis of lncRNA and other non-coding RNA. The kit additionally provides digestion probes targeting mitochondrial rRNA and chloroplast rRNA, enabling it to efficiently remove major rRNAs from a variety of plant species and provide the most informative sequencing results. 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.

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_2O	1 ml	2 × 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 (Plant) is suitable for removing rRNA (including 5S, 18S, and 25S rRNA) from the total RNA of plant roots, seeds, and leaves with an initial template amount of 1 - 5 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 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, low-adsorption EP tubes (Eppendorf #022431021); Agilent 2100 Bioanalyzer or other equivalent products, PCR instrument, magnetic separation rack, etc.

06/Notes

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

06-1/About Storage

a. 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.

b. 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

a. Do not leave the RNA on the ice for a long time after dilution to 10 μl or 11 μl with RNase-free ddH_2O to avoid RNA degradation.

b. 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 (using VAHTS RNA Clean Beads, Vazyme #N412), or other methods.

06-3/About Magnetic Beads

a. The magnetic beads should be equilibrated to room temperature after being taken out from the 2 ~ 8℃ environment to ensure optimal capture efficiency.

b. The magnetic beads should be thoroughly mixed by vortexing every time before pipetting.

c. 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.

d. When purifying the ribosomal-depleted RNA, make sure to use 80% ethanol freshly prepared with RNase-free ddH_2O to rinse the magnetic beads; otherwise, the RNA may be degraded, leading to library preparation failure.

e. In the second rinse of the magnetic beads with 80% ethanol, do not leave any supernatant to reduce residual impurities.

f. The beads should be dry enough before elution (the surface changes from glossy brown to matte 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

a. It is recommended to use filter pipette tips; change tips when pipetting different samples.

b. Always wear gloves; change gloves after contact with the equipment outside the RNase-free area or entering other work areas.

c. All reagents must be capped immediately after use to avoid contamination.

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

07/Mechanism & Workflow

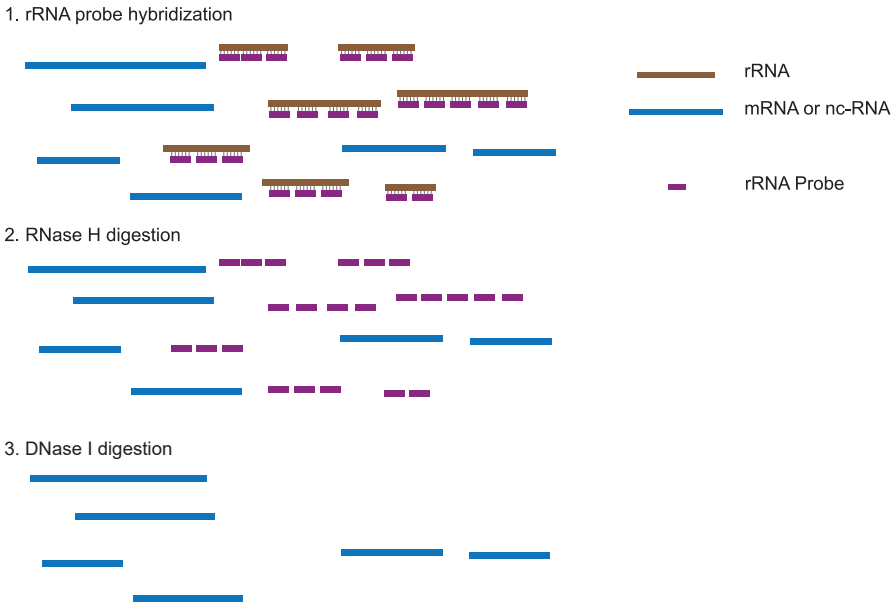

Fig 1. Schematic Diagram of rRNA Depletion

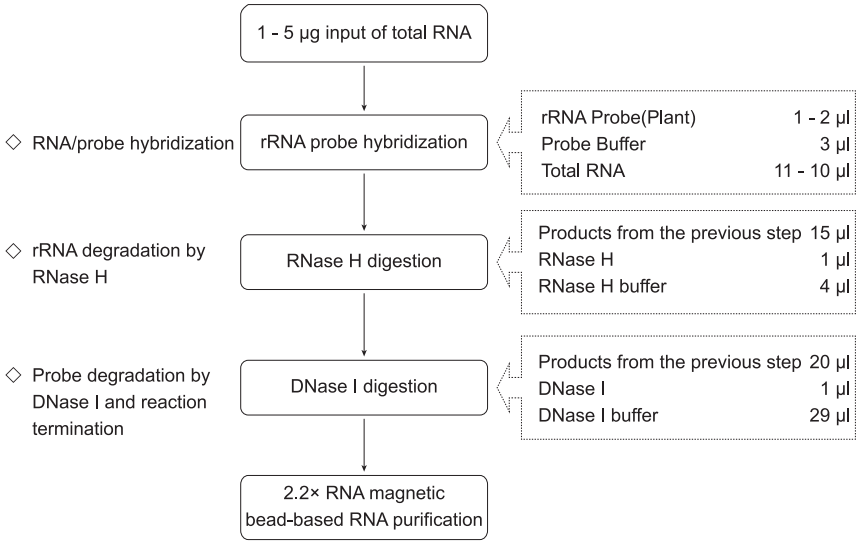

Fig 2. Workflow of rRNA Depletion

08/Experiment Process

08-1/Probe Hybridization

1. Prepare the total RNA sample: Dilute 1 - 5 μg of total RNA with Nuclease-free ddH_2O in a Nuclease-free centrifuge tube to 10 μl or 11 μl and keep the tube on ice for later use.

   ▲ The components needed for the next step can be taken out from the -20℃ storage in advance and placed on ice for later use.

2. Prepare the following reaction mix in a Nuclease-free centrifuge 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	

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

   ▲ If multiple samples are processed simultaneously, a mixture of rRNA Probe (Plant) 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 to allow probe hybridization.

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

   ▲ This step takes about 15 - 20 min, which may vary between different models of PCR instruments.

   ▲ The components needed for the next step can be taken out from the -20℃ storage in advance and placed on ice for later use.

08-2/RNase H Digestion

1. 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	

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

   ▲ If multiple samples are processed simultaneously, a mixture of RNase H Buffer and RNase H 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.

2. Load the sample into the PCR instrument and run the following program for RNase H digestion.

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

   ▲ The components needed for the next step can be taken out from the -20℃ storage in advance and placed on ice for later use.

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. Collect the solution to the bottom of the tube by brief centrifugation.

▲ If multiple samples are processed simultaneously, a mixture of RNase H Buffer and RNase H 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.

2. Load the sample into the PCR instrument and run the following program for RNase H digestion.

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

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/Purification of Ribosomal-Depleted RNA

1. Mix the VAHTS RNA Clean Beads thoroughly by vortexing. Pipette 110 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. Keep the sample on the magnetic rack for 5 min. After the solution becomes clear (about 5 min), carefully remove the supernatant.
4. Keep the sample on the magnetic rack. 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 rack. Uncap the tube and air-dry the beads at room temperature for 5 - 10 min.
   ▲ Do not re-suspend 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.

7-1. If the purified product is used for reverse transcription, remove the sample from the magnetic rack, 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 rack 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 -80 ~ -65℃ for later use.

7-2. 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 rack, add 18.5 μl of 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 rack for 5 min. After the solution becomes clear, carefully transfer 16 μl of the supernatant into a new Nuclease-free PCR tube. Prepare the library immediately.

09/FAQ & Troubleshooting

◇ 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.

◇ 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℃.

◇ 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 starting 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 5 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.

Vazyme