Ribo-off rRNA depletion Kit (Plant)

NB-54-0059-01
NB-54-0059-02

Ribo-off rRNA depletion Kit (Plant)

Cat# NB-54-0059-01 size: 12 rxns

Cat# NB-54-0059-02 size: 24 rxns

01/Product Description

The Ribo-off rRNA depletion kit (Plant) is designed to deplete rRNA (including 5S, 18S and 25S rRNA) from total RNA of plant roots, seeds and leaves and to obtain mRNA and other non-coding RNA. This kit is suitable for removing rRNA from 1 μg - 5μg of plant total RNA. rRNA is depleted by rRNA-toprobe hybridization, RNase H digestion, and DNaseI digestion steps. The retained mRNA and other non-coding RNA can be used for LncRNA and other non-coding RNAs analysis.

This kit contains digestion probes targeting of mitochondrial rRNA and chloroplast rRNA. It can efficiently remove major rRNAs from a variety of plant species and provide the most informative sequencing results. This kit is applicable for both intact and degraded RNA samples. The resulting rRNA-depleted RNA is suitable for RNA library construction and other downstream experiments.

02/Components

Components	NB-54-0059-01 (12 rxn)	NB-54-0059-02 (24 rxn)
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 ddH2O	1 ml	2 × 1 ml

03/Storage

All the components should be stored at -30℃ ~ -20℃, transported at -20℃ ~ 0℃.

04/Applications

The Ribo-off rRNA depletion kit (Plant) is applicable to deplete rRNA (including 5S, 18S and 25S rRNA) from 1 μg - 5μg total RNA of plant roots, seeds and leaves and to obtain mRNA and other non-coding RNA. This kit is suitable for both intact and degraded RNA samples.

The mRNA content of total RNA varies in different samples. The initial input of total RNA should be appropriately adjusted according to the downstream application. The resulting rRNA-depleted RNA can be used for transcriptome library construction with strand-specific information or non-strand-specific.

05/ Additional Materials Required

- ◇ RNA Analysis: Agilent RNA 6000 Pico Kit (Agilent #5067-1513);
- ◇ RNA Clean Beads: VAHTS RNA Clean Beads (NB-54-0061) or Agencourt RNA Clean XP Beads (Beckman, #A63987);
- ◇ Other Materials: 80% Ethanol (freshly prepared using Nuclease-free ddH2O), Nuclease-free ddH2O, Nuclease-free PCR tubes, Low absorption EP tubes (Eppendorf, #022431021), Agilent 2100 Bioanalyzer, Thermocycler (PCR instrument), Magnetic stand.

06/ Notes

06-1/ All components should be stored as labeling conditions.

- ◇ The kit contains various Enzymes and must be stored at -30°C ~ -15°C. It should be placed on ice during use and stored at -30°C ~ -15°C immediately after using, otherwise the activity of the enzyme may be reduced.
- ◇ It is recommended to dispense the reagents into several portions after the first use, avoiding repeated freezing and thawing.

06-2/ RNA Sample processing.

- ◇ After diluting total RNA into 11 µl with Nuclease-free H2O, Do not place the RNA on ice for a long time avoiding RNA degradation.
- ◇ For the total RNA with low concentration resulting in volume > 11 µl, it can be concentrated using lyophilization, ethanol-precipitation, column-based or bead-based clean-up (e.g. VAHTS RNA Clean Beads, Neo Biotech # NB-54-0061).

06-3/ Select the correct fragmentation programs and the appropriate number of amplification cycles.

If the resulting rRNA-depleted RNA is used for transcriptome library construction, the recommended fragmentation condition is 85°C for 6 min, amplification cycle is 15 cycles, and size selection condition is 0.65× / 0.1×.

06-4/ RNA Purification with Magnetic Beads.

- ◇ Equilibrate the beads to room temperature before use to assure capture efficacy. ◇Mix the beads thoroughly every time before pipetting.
- ◇ Transfer the supernatant after the solution is completely clarified. Keep tubes on magnet stand without disturbing the beads.
- ◇ It is necessary to prepare 80% ethanol freshly with Nuclease-free H2O to rinse the RNA purification beads. Otherwise, it will cause RNA degradation, resulting in library construction failure.
- ◇ Do not leave any 80% ethanol supernatant behind in the second washing step to reduce the residual impurities.
- ◇ It is important to remove all the ethanol before proceeding with subsequent reactions. However, over-drying of beads may make them difficult to resuspend, resulting in a dramatic loss of RNA.

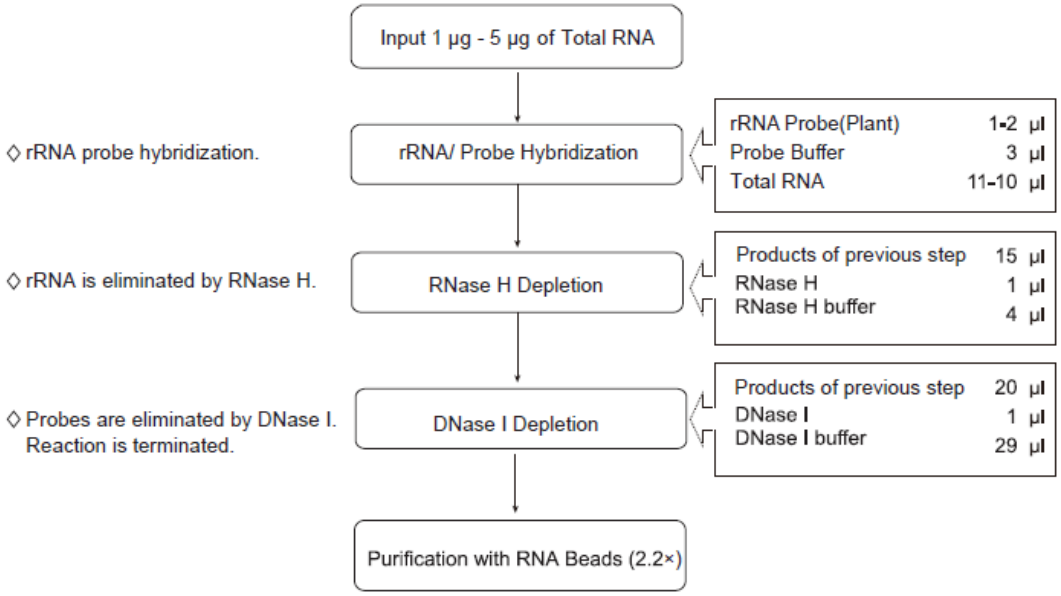
06-5/ Operational Attentions.

- ◇ It is recommended to use filter pipette tips; change tips when pipetting different samples.
- ◇ Be sure to wear gloves; change gloves after touching the equipment outside the RNase-free space or other working areas.
- ◇ Please cover the reagent with lid to avoid contamination whenever finish use.
- ◇ The enzyme components should be briefly centrifuged before use to avoid sticking to the wall and lid of tube, resulting in loss.

07/ Mechanism and Workflow

Mechanism

Workflow



08/ Protocol

08-1/ rRNA/Probe hybridization

1. Prepare total RNA samples: Dilute 1 µg - 5 µg of total RNA with 11 µl or 10 µl of Nuclease-free Water in a Nuclease-free PCR tube and keep the tube on ice for use.
   ▲The components for next step can be taken out from -30°C ~ -15 °C in advance and placed on ice for use.
2. Prepare the following reaction solution in a Nuclease-free centrifuge tube:

Components	1 - 2.5 µg of Input RNA	2.5 - 5 µg of Input RNA
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 thoroughly by gently pipetting up and down for 10 times. Collect the liquid to the bottom of the tube by a brief centrifugation.
   ▲ For multiple samples, it is recommended to prepare a mixture of rRNA Probe (Plant) and Probe Buffer in a suitable size tube first, and then dispense into each PCR tube.
   It is recommended to prepare 1.1 x volumes of the actual volume to compensate for the loss.
3. Put the sample into a PCR instrument and run the following program:

Procedure	Temperature	Time
Hot Lid	105°C	on
RNA Denaturation	95°C	2 min
Probe Hybridization	95°C - 22°C	0.1°C/sec
Incubation	22°C	5 min
Hold	4°C	Hold

   ▲ This step takes approximately 15 min - 20 min, which may vary between different PCR instruments.
   ▲ The components for next step can be taken out from -30°C ~ -15 °C in advance and placed on ice for use.

08-2/ Digestion with RNase H

1. Prepare the following reaction solution on ice:

Components	Volume	
RNase H Buffer	4 µl	■
RNase H	1 µl	■
Products of Step 08-1	15 µl	
Total	20 µl	

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

▲For multiple samples, it is recommended to prepare a mixture of RNase H Buffer and RNase H in a suitable size tube first, and then dispense into each PCR tube. It is recommended to prepare 1.1 x volumes of the actual volume to compensate for the loss.

2. Put the sample into a PCR instrument and run the following program for RNase H digestion reaction:

Procedure	Temperature	Time
Hot Lid	105˚C	on
RNase H Digestion	37˚C	30 min
Hold	4˚C	

08-3/ Digestion with DNase I

1. Prepare the following reaction solution on ice:

Components	Volume	
DNase I Buffer	29 µl	■
DNase I	1 µl	■
RNase H Digested Products (Products of Step 08-2)	20 µl	
Total	50 µl	

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

▲For multiple samples, it is recommended to prepare a mixture of DNase I Buffer and DNase I in a suitable size tube first, and then dispense into each PCR tube. It is recommended to prepare 1.1 x volumes of the actual volume to compensate for the loss.

2. Put the sample into a PCR instrument and run the following program for DNase I digestion reaction:

Procedure	Temperature	Time
Hot Lid	105˚C	on
DNase I Digestion	37˚C	30 min
Hold	4˚C	

3. Collect the liquid to the bottom of the tube by a brief centrifugation. Put the tube on ice and immediately proceed to the next procedure.

08-4/ Purification of Ribosomal-depleted RNA with VAHTS RNA Clean Beads.

1. Suspend the VAHTS RNA Clean Beads thoroughly by vortexing, pipet 110 µl (2.2 ×) of beads into the RNA sample of Step 08-3. Mix thoroughly by pipetting up and down for 10 times.
2. Incubate the sample on ice for 15 min to make the RNA bind to the beads.
3. Put the sample onto a magnetic stand. Wait until the solution clarifies (about 5 min). Then carefully discard the supernatant without disturbing the beads.
4. Keep the sample on the magnetic stand, add 200 µl of 80% Ethanol (freshly prepared using Nuclease-free ddH2O) to rinse the beads. Incubate at room temperature for 30 sec and carefully discard the supernatant without disturbing the beads.
5. Repeat **Step 4.**
6. Keep the sample on the magnetic stand, open the tube and air-dry the beads for 5 min - 10 min.

7. Option A: (if the ribosomal-depleted RNA will be used for reverse transcription): take the sample out of magnetic stand, add 20 µl of Nuclease-free ddH2O and mix thoroughly by pipetting for 6 times, and incubate at room temperature without shaking for 2 min. Put the tube back on the magnetic stand and wait until the solution clarifies (about 5 min), carefully transfer 18 µl of the supernatant to a new Nuclease-free PCR tube without disturbing the beads.
8. The eluted Ribosomal-depleted RNA is now ready for reverse transcription or RNA library preparation or storage at -70℃.

Troubleshooting

◇ If the purified product is used for library construction, but use Nuclease-free H2O for elution by mistake.
1. If condition permits, add an equal volume of 2 × Frag/Prime Buffer (Vazyme #N402). And the volume in subsequent reaction system should be amplified according the ratio until the purification step is completed. Then restore the original volume in reaction system.
2. Using VAHTS RNA Clean Beads (Neo Biotech NB-54-0061-01) to purify again, and finally elute with Frag/Prime Buffer.

◇ How long can the purified product be stored?
The purified product is easily degraded due to its low concentration.
It is recommended to proceed to the next procedures immediately after eluting Ribosomal-depleted RNA. Otherwise, store Ribosomal-depleted RNA at -70°C.

◇ Methods to solve the problem of low concentration of library.
It is recommended to use high-quality RNA samples as templates for library construction to make library concentration meet the requirements of the machine sequencing. If you cannot provide qualified RNA samples, try to use the following methods to make up:
1. Initial amount: Increase the initial amount up to 5 µg.
2. Do Prepare several duplicate samples, merge them after the Fragmentation step, or before PCR step.
3. Take option without size selection: Though RNA fragmented at 94°C for 8 min is short, its distribution is concentrated and the homogeneity is also well. However, some individualized samples have non-uniform fragments and this situation will be amplified by PCR, but this situation rarely occurs in the option of without size selection.