Hyaseq rRNA Depletion Kit (Plant)

Cat.No.: HYD219

Product Description

Hyaseq rRNA Depletion Kit (Plant) adopts the RNase H digestion method to remove ribosomal RNA (rRNA) from plant total RNA, including cytoplasmic 18S & 25S rRNA, mitochondrial 18S & 26S rRNA, and chloroplast 16S & 23S rRNA, while retaining messenger RNA (mRNA) and other non-coding RNAs. This kit delivers efficient rRNA depletion for both intact and partially degraded total RNA. The rRNA-depleted RNA samples are suitable for high-throughput sequencing of mRNA and non-coding RNAs, which can greatly increase the proportion of valid sequencing reads. The obtained RNA can also be applied to cDNA synthesis and other downstream experiments.

Components

Components	24 T	96 T
Hybridization Buffer	72 µL	288 µL
Plant Probe Mix	72 µL	288 µL
RNase H Buffer	72 µL	288 µL
Thermostable RNase H	48 µL	192 µL
DNase I Buffer	660 µL	2×1320 µL
DNase I	60 µL	240 µL

Storage

Store at -25 ~ -15 ℃, valid for 1 year.

Notes

1. Use RNase-free consumables and regularly decontaminate the work area. RNAZap™ (Thermo Fisher) is recommended for efficient RNase removal.
2. Ensure RNA samples are free of genomic DNA (gDNA). If gDNA contamination exists, perform DNase I digestion and purification prior to using this kit.
3. The maximum loading volume of RNA sample is 10 µL. Concentrate the sample if its volume exceeds the limit.
4. Prepare RNase H Mix freshly right before use.

5. Wear lab coat and disposable gloves during operation for personal protection.
6. For Research Use Only.

Required Materials

1. RNA purification magnetic beads: NGS RNA Clean Beads (Cat.No.: HYD513) or equivalent products.
2. qRT-PCR reagents for rRNA depletion efficiency detection: 2X Universal SYBR Green Fast qPCR Mix (Cat.No.: HYA412) or equivalent products.
3. Other supplies: Absolute ethanol, nuclease-free ultrapure water, low-retention pipette tips, PCR tubes, magnetic stand, thermal cycler, etc.

Instructions

1. Probe Hybridization

1) Take probes and Hybridization Buffer from -20 °C, thaw, mix thoroughly by inverting, and place on ice for later use.
2) Prepare RNA samples: Calculate the required RNA volume based on input amount and sample concentration, then dilute to 9 μL with nuclease-free water.
3) Prepare the rRNA depletion reaction mixture in 200 μL PCR tubes according to Table 1.

Table 1. Probe Hybridization Reaction System

Components	Volume
Hybridization Buffer	3 μL
Plant Probe Mix	3 μL
Total RNA	9 μL (100 ng–1 μg)

4) Gently pipette to mix, then spin down briefly to collect all liquid at the tube bottom.
5) Place the PCR tubes in a thermal cycler and run the probe hybridization program as specified in Table 2.

Table 2. Probe Hybridization Program

Temperature	Time
Heat Lid 105 °C	On
95 °C	2 min
95 °C - 42 °C	0.1°C/s
42 °C	5 min
4 °C	hold

2. RNase H Digestion

1) Thaw RNase H digestion reagents retrieved from -20 °C, mix by inverting and keep on ice. Prepare the RNase H digestion reaction mixture following Table 3.

Table 3. RNase H Digestion Reaction System

Components	Volume
RNase H Buffer	3 μL
Thermostable RNase H	2 μL
Previous reaction product	15 μL

Note: Add RNase H Buffer and RNase H separately. If a master mix needs to be prepared for large batches of samples, make it freshly before use. Improper preparation will compromise rRNA depletion efficiency.

2) Gently pipette to mix, then spin down briefly to collect liquid at the tube bottom.

3) Place the PCR tubes in a thermal cycler and run the program as follows: Heat lid at 75 °C; 65 °C for 30 min; hold at 4 °C to complete RNase H digestion.

3. DNase I digestion

1) Thaw DNase I digestion reagents from -20 °C, thaw, mix by inverting and keep on ice. Prepare the DNase I digestion reaction mixture as shown in Table 4.

Table 4. DNase I Digestion Reaction System

Components	Volume
DNase I Buffer	27.5 μL
DNase I	2.5 μL
Previous reaction product	20 μL

2) Gently pipette to mix, then spin down briefly to collect all liquid at the tube bottom.

3) Place the PCR tubes in a thermal cycler and run the program: Heat lid at 50 °C; 37 °C for 30 min; hold at 4 °C for DNase I digestion.

4. RNA Purification

1) Preparation: Take out NGS RNA Clean Beads (Cat.No. HYD513) and equilibrate to room temperature for no less than 30 min. Prepare 80% ethanol with nuclease-free water.
2) Vortex or invert thoroughly to fully resuspend the beads.
3) Add 110 μL NGS RNA Clean Beads (2.2×, Beads:RNA = 2.2:1) to the sample from the previous step. Mix thoroughly by pipetting, then incubate at room temperature for 5 min.
4) Place the PCR tube on a magnetic stand for separation. After the solution turns clear (approx. 3 min), carefully remove the supernatant.
5) Keep the tube on the magnetic stand. Add 200 μL freshly prepared 80% ethanol (prepared with nuclease-free water) to wash the beads. Incubate at room temperature for 30 sec, then carefully discard the supernatant.
6) Repeat step 4.5 for a second wash. Remove residual liquid completely using a 10 μL pipette.
7) Keep the tube on the magnetic stand and leave the lid open to air-dry the beads at room temperature for 5–10 min.
8) RNA Elution: Remove the tube from the magnetic stand. Add 11 μL nuclease-free water (or an appropriate volume of nuclease-free water/elution buffer for downstream applications). Gently pipette to mix well and incubate at room temperature for 5 min.
9) Briefly spin down the tube and place it back on the magnetic stand. Once the solution clarifies (approx. 3 min), carefully transfer 10 μL supernatant to a new nuclease-free PCR tube. The transfer volume can be adjusted according to the actual elution volume used in Step 4.7.

Note: Use the eluted sample immediately for downstream experiments, or store at -80 °C.