Ribo-off rRNA Depletion Kit (Human/Mouse/Rat)

N406

Version 22.1

Product Description

Ribo-off rRNA Depletion Kit (Human/Mouse/Rat) is designed for removing ribosomal RNA (rRNA) (including cytoplasmic 28S, 18S, 5.8S, 5S rRNA, and mitochondrial 16S, 12S rRNA) from total RNA of humans, mice, and rats while retaining mRNA and other non-coding RNA. The kit is applicable to both intact and partially degraded RNA samples (e.g. FFPE RNA), and the resulting ribosomal-depleted RNA can be used for the analysis of mRNA and non-coding RNA (such as lncRNA).

Components

	Components	N406-01 (24 rxns)	N406-02 (96 rxns)
■	rRNA Probe (H/M/R)	24 μl	96 μl
■	Probe Buffer	72 μl	288 μl
■	RNase H Buffer	96 μl	384 μl
■	RNase H	24 μl	96 μl
■	DNase I Buffer	696 μl	4 × 696 μl
■	DNase I	24 μl	96 μl
□	Nuclease-free ddH_2O	1 ml	4 × 1 ml

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Self-prepared Materials

Magnetic stand

Ethanol absolute

Nuclease-free PCR tube

VAHTS RNA Clean Beads (Vazyme #N412)

Applications

Initial template: 0.1 - 1 μg total RNA from humans, mice, or rats.

Notes

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

1. To ensure rRNA depletion efficiency, RNA samples should be free of salt ions (such as Mg^{2+} or guanidine salts) and organic compounds (such as phenol and ethanol).
2. To avoid DNA contamination, RNA samples can be treated with DNase I to remove trace DNA.
3. The ribosomal-depleted RNA yield depends on the quality of initial RNA, the content of rRNA in the sample, and the purification method used, with a general recovery of 3% - 10%.
4. For RNA-seq, it is recommended to start with over 100 ng of input total RNA to increase library complexity.

Experiment Process

1. Total RNA sample preparation

Dilute 0.1 - 1 μg of total RNA with Nuclease-free ddH_2O in an Nuclease-free PCR tube to 11 μl and keep the tube on ice for later use.

2. Probe hybridization

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

Components	Volume	
rRNA Probe (H/M/R)	1 μl	■
Probe Buffer	3 μl	■
Total RNA	11 μl	
Total	15 μl	

b. Mix well by gently pipetting up and down, and collect the sample to the bottom of the tube by instantaneous centrifugation.

c. Load the sample into the PCR instrument. Run the following program, which takes approximately 15 - 20 min.

Temperature	Time
95℃	2 min
95 ~ 22℃	0.1℃/sec
22℃	5 min

d. Collect the sample to the bottom of the tube by instantaneous centrifugation, place the tube on ice, and proceed to the next step immediately.

3. RNase H digestion

a. 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

b. Mix well by gently pipetting up and down, and collect the sample to the bottom of the tube by instantaneous centrifugation.

c. Load the sample into the PCR instrument, and incubate at 37℃ for 30 min.

d. Collect the sample to the bottom of the tube by instantaneous centrifugation, place the tube on ice, and proceed to the next step immediately.

4. DNase I digestion

a. Prepare the following reaction mix on ice.

Components	Volume
DNase I Buffer	29 µl
DNase I	1 µl
Products after RNase H digestion	20 µl
Total	50 µl

b. Mix well by gently pipetting up and down, and collect the sample to the bottom of the tube by instantaneous centrifugation.

c. Load the sample into the PCR instrument, and incubate at 37℃ for 30 min.

d. Collect the sample to the bottom of the tube by instantaneous centrifugation, place the tube on ice, and proceed to the next step immediately.

5. Purification of ribosomal-depleted RNA with VAHTS RNA Clean Beads

a. 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.

b. Incubate the sample on ice for 15 min to allow the RNA to bind to the magnetic beads.

c. Hold the sample on the magnetic stand for 5 min. After the solution becomes clear, carefully remove the supernatant.

d. Keep the sample on the magnetic stand. Add 200 µl of 80% ethanol freshly prepared with Nuclease-free ddH_2O to rinse the beads (do not re-suspend the beads). Incubate the sample at room temperature for 30 sec and carefully remove the supernatant.

e. Repeat the previous step for a total of two rinses.

f. Keep the sample on the magnetic stand. Uncap the tube and air-dry the beads at room temperature for 5 - 10 min.

- ◇ If the purified product is used for reverse transcription, remove the sample from the magnetic stand, add 10.5 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. Hold the sample on the magnetic stand for 5 min. After the solution becomes clear, carefully transfer 8 µl of the supernatant into a new Nuclease-free PCR tube.
- ◇ If the purified product is used for library preparation with VAHTS Universal V8 RNA-seq Library Prep Kit for Illumina (Vazyme #NR605), remove the sample from the magnetic stand, add 18.5 µl of 1 × Frag/Prime Buffer, mix thoroughly by pipetting up and down 6 times, and allow to stand at room temperature for 2 min. Hold the sample on the magnetic stand 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.

g. Use the sample immediately for RNA-seq library preparation or other analytical applications or storage at -80 ~ -65℃ for later use.