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

Catalog # N406

Version 5.1

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Introduction

The Ribo-off rRNA Depletion Kit (H/M/R) is designed to deplete rRNA (including cytoplasmic 28S, 18S, 5S rRNA, and mitochodrial 12S, 5.8S rRNA) from human, mouse, and rat total RNA preparations, while leaving mRNA and other non-coding RNA. This kit is suitable for both intact and degraded RNA samples (i.e. FFPE RNA). The obtained rRNA-depleted RNA can be used for analysis applications of mRNA and non-coding RNA (e.g. lncRNA).

Contents of Kit

Components	N406-01 (24rxn)	N406-02 (96rxn)
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 Water	1 ml	4 ml

Storage

All the components should be stored at -20℃.

Additional Materials Required

Magnetic Stand

100% Ethanol

Nuclease-free PCR Tube

VAHTS RNA Clean Beads (Vazyme, #N412) or Agencourt® RNAClean® XP Beads (Beckman Coulter, #A63987).

Application

Starting Materials: 0.1 µg-1 µg of human, mouse, or rat total RNA.

Protocol

1. Preparation of total RNA sample

1.1. Dilute 0.1 µg-1 µg of total RNA with 11 µl of Nuclease-free Water in a Nuclease-free PCR tube and keep on ice.

2. rRNA/Probe hybridization

2.1. Prepare the following reaction solution in a Nuclease-free PCR tube:

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

Mix by gently pipetting up and down for 10 times.

2.2. Collect the liquid to the bottom of the tube by a brief centrifugation. Put the sample into a PCR instrument and run the following program (Hot Lid Temperature: 105℃):

95℃	2 min
95-22℃	0.1℃ /sec
22℃	5 min

3. Digestion with RNase H

3.1. Prepare the following reaction solution on ice:

RNase H Buffer	4 µl
RNase H	1 µl
Products of **Step 2.2**	15 µl
Total	20 µl

Mix by gently pipetting up and down for 10 times.

3.2. Place the sample in a PCR instrument and incubate at 37℃ for 30 min (Hot Lid Temperature: 105℃).

4. Digestion with DNase I

4.1. Prepare the following reaction solution on ice:

DNase I Buffer	29 μl
DNase I	1 μl
RNase H Digested Products	20 μl
Total	50 μl

Mix by gently pipetting up and down for 10 times.

4.2. Place the sample in a PCR instrument and incubate at 37℃ for 30 min (Hot Lid Temperature: 105℃). 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.

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

5.1. Suspend the **VAHTS RNA Clean Beads** thoroughly by vortexing, pipet 110 μl (2.2×) of beads into the RNA sample of **Step 4.2**. Mix thoroughly by pipetting fup and down for 10 times.

5.2. Incubate the sample on ice for 15 min to make the RNA bind to the beads.

5.3. Put the sample onto a magnetic stand. Wait until the soultion clarifies (about 5 min). Then carefully discard the supernatant without disturbing the beads.

5.4. Keep the sample on the magnetic stand, add 200 μl of **freshly prepared 80% ethanol** to rinse the beads. **DO NOT re-suspend the beads!** Incubate at room temperature for 30 sec and carefully discard the supernatant without disturbing the beads.

Note: It is highly recommended to use a 10 μl pipette to remove the residual supernatant in this step.

5.5. Repeat **Step 5.4**.

5.6. Keep the sample on the magnetic stand, open the tube and air-dry the beads for 5 min-10 min.

5.7A. (**Option A**) If the Ribosomal-depleted RNA will be used for reverse transcription: Take the sample out of magnetic stand. Add 10.5 μl of Nuclease-free Water and mix thoroughly by pipetting for 6 times. Incubate at room temperature without shaking for 2 min. Then, put the tube back on the magnetic stand and wait until the soultion clarifies (about 5 min) . Carefully transfer 8 μl of the supernatant to a new Nuclease-free PCR tube without disturbing the beads.

5.7B. (**Option B**) If the Ribosomal-depleted RNA will be used for RNA library preparation with VAHTS Total RNA-seq (H/M/R) Library Prep Kit for Illumina® (Vazyme, #NR603): Take the sample out of magnetic stand. Add 18.5 μl of Frag/Primer Buffer and mix thoroughly by pipetting up and down for 6 times. Incubate at room temperature without shaking for 2 min. Put the tube back on the magnetic stand and wait until the soultion clarifies (about 5 min) . Carefully transfer 16 μl of the supernatant to a new Nuclease-free PCR tube without disturbing the beads.

5.8. The eluted Ribosomal-depleted RNA is now ready for reverse transcription or RNA library preparation. It is highly recommended to proceed to the next procedures immediately, rather than to store the RNA at -20℃.

Notes

1. To ensure the removal efficiency of rRNA, the RNA samples should be free of salt ions (i.e. Mg^{2+} or guanidine salts) and organic compounds (i.e. phenol and ethanol).
2. The DNase I treatment is to to remove trace amounts of DNA and thereby to avoid DNA contamination of DNA.
3. The yield of rRNA-depleted RNA depends on the quality of the starting RNA, the rRNA content in the sample, and the purification method used. The average yield rate is 3%-10%.
4. For RNA-Seq samples, to increase library complexity, it is recommended to use total RNA with an amout of > 100 ng as starting materials.