Ribo-off rRNA Depletion Kit (Bacteria)

Catalog # N407

Version 8.0

Vazyme biotech co., ltd.

Introduction

The Ribo-off rRNA Depletion Kit (Bacteria) is designed to deplete rRNA (including 16S and 23S rRNA) from total RNA of Gram-positive and Gram-negative bacteria and to obtain mRNA and other non-coding RNA. This kit is suitable for both intact and degraded RNA samples (i.e. FFPE RNA) and can remove rRNA in total RNA of 1 μg - 5 μg. The obtained rRNA-depleted RNA can be used for analysis applications of mRNA and non-coding RNA (i.e. lncRNA) and other applications.

Contents of Kit

Components	N407-01 (12 rxn)	N407-02 (24 rxn)
rRNA Probe (Bacteria)	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

Storage

All the components should be stored at -20℃.

Additional Materials Required

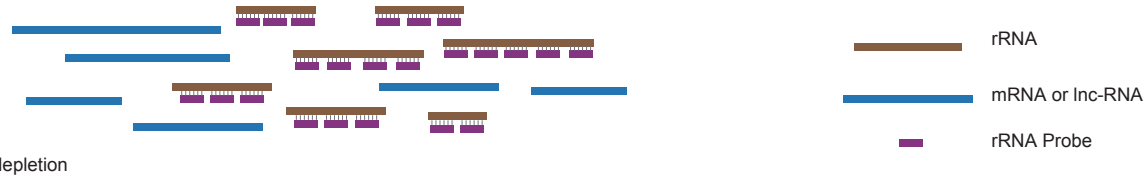
RNA Analysis: Agilent RNA 6000 Pico Kit (Agilent #5067-1513)

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

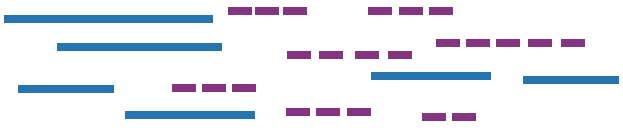
Other Materials: Magnetic Stand, 80% Ethanol (freshly prepared using Nuclease-free ddH2O), Nuclease-free ddH2O, Nuclease-free PCR tubes, Low absorption EP tubes (Eppendorf, #022431021), Agilent 2100 Bioanalyzer, Thermocyler (PCR instrument), Magnetic stand.

Mechanism

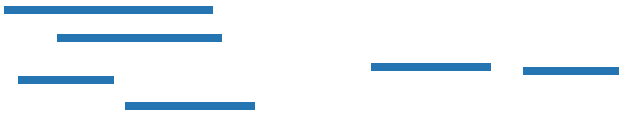
1. rRNA probe hybridization



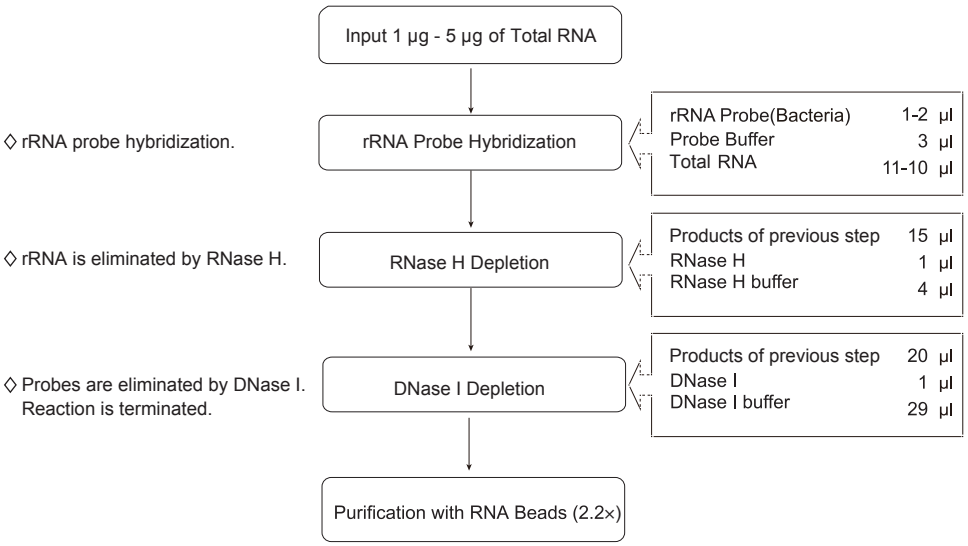
2. RNase H depletion



3. DNase I depletion



Workflow



Protocol

1. Hybridization of RNA sample and probe

1.1. In a Nuclease-free PCR tube, dilute 1 μg - 5 μg of total RNA in 10 μl or 11 μl of Nuclease-free ddH2O. Then, prepare the following reaction solution:

	1 μg - 2.49 μg of Input RNA	2.5 μg - 5 μg of Input RNA
rRNA Probe (Bacteria)	1 μl	2 μl
Probe Buffer	3 μl	3 μl
Total RNA	11 μl	10 μl
Nuclease-free ddH2O	To 15 μl	To 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.

1.2. Put the sample into a PCR instrument and run the following program:

	Hot Lid, 105℃	On
RNA Denaturation	95℃	2 min
Probe Hybridization	From 95℃ to 22℃	0.1℃ / sec
Incubation	22℃	5 min
	4℃	Hold

▲ This step takes approximately 15 min - 20 min, which may vary between different PCR instruments.
▲ Take all the necessary components out of the -20℃ refrigerator and put them on ice before use.

2. Digestion with RNase H

2.1. Prepare the following reaction solution on ice:

RNase H Buffer	4 μl
RNase H	1 μl
Products of **Step 1.2.**	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.

2.2. Load the sample in a PCR instrument and run the following program:

	Hot Lid, 105℃	On
RNase H Depletion	37℃	30 min
	4℃	Hold

▲ Take all the necessary components out of the -20℃ refrigerator and put them on ice before use.

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

3. Digestion with DNase I

3.1. Prepare the following reaction solution on ice:

DNase I Buffer	29 µl	■
DNase I	1 µl	■
RNase H Digested Products (Products of **Step 2.3.**)	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.

3.2. Load the sample in a PCR instrument and run the following program:

	Hot Lid, 105℃	On
DNase I Depletion	37℃	30 min
	4℃	Hold

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

4 Purification of Ribosomal-depleted RNA with VAHTS RNA Clean Beads

4.1. Suspend the VAHTS RNA Clean Beads thoroughly by vortexing, pipet 110 µl (2.2 ×) of beads into the RNA sample of **Step 3.3.** Mix thoroughly by pipetting up and down for 10 times.

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

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

4.4. Keep the sample on the magnetic stand, add 200 µl of 80% Ethanol (**freshly prepared using Nuclease-free ddH$_2$O**) to rinse the beads. Incubate at room temperature for 30 sec and carefully discard the supernatant without disturbing the beads.

4.5. Repeat **Step 4.4.**

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

▲ **DO NOT** re-suspend the beads when adding 80% ethanol to rinse.

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

▲ Avoid over dry in case of decreasing the recovery efficiency of RNA.

4.7-**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 ddH$_2$O 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.

4.7-**Option B** (if the ribosomal-depleted RNA will be used for RNA library preparation with VAHTS Stranded mRNA-seq Library Prep Kit for Illumina® V2) (Vazyme, #NR612): 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, 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 16 µl of the supernatant to a new Nuclease-free PCR tube without disturbing the beads for library preparation.

▲ Recommended fragmentation condition: 85℃, 6 min. Recommended size-selection condition: 0.65 × / 0.1 ×.

▲ Recommended amplification cycle numbers: 15.

4.8. The eluted Ribosomal-depleted RNA is now ready for reverse transcription or RNA library preparation or storage at -20℃.

▲ It is highly recommended to proceed to the next procedures immediately.

Appendix

Note: The species below that have been tested can be applied to Ribo-off rRNA Depletion Kit (Bacteria). But this kit is not limited to the below species.

Ribo-off rRNA Depletion Kit (Bacteria) Species Compatibility:					
1	Acanthamoeba	39	*Metallosphaera sedulla*	77	*Toxoplasma gondii*
2	*Acinetobacter baumanii*	40	*Methanobacterium*	78	*Aeromonas hydrophila*
3	*Actinoplanes spp*	41	*Methanococcus maripaludis*	79	*Xanthomonas campestris*
4	*Amoebophilus asiaticus*	42	*Methanolobus psychrophillus*	80	*Janthinobacterium svalbardensis*
5	*Arthroabcter arilaitensis Re117*	43	*Methanoseata concilii*	81	*Lactococcus lactis*
6	*Azotobacter vinelandii*	44	*Microcystis aeruginosa*	82	*Corynebacterium glutamicum*
7	*Bacillus subtilis*	45	*Moraxella catarrhalis*	83	*Burkholderia*
8	*Bacteroides vulgatus*	46	*Mycobacterium tuberculosis*	84	*Bacillus cereus*
9	*Brucella abortus*	47	*Mycobacterium*	85	*Escherichia coli*
10	*Bartonella henselae*	48	*Paratuberculosis*	86	*Streptomyces coelicolor*
11	*Borrelia burgdorferi*	49	*Mycoplasma gallisepticum*	87	*Vibrio alginolyticus*
12	*Brevibacterium aurantiacum*	50	*Mycoplasma mycoides*	88	*Azotobacter sp.*
13	*Burkholderia pseudmoallei*	51	*Nitrospira*	91	*Staphylococcus aureus*
14	*Campylobacter jejuni*	52	*Pantoea agglomerans*	92	*Pseudomonas aeruginosa*
15	*Chromohalobacter*	53	*Prevotella copri*	93	*Lactobacillus plantarum*
16	*Clostridium difficile*	54	*Photorhabdus*	94	*Bacillus licheniformis*
17	*Clostridium ljungdahlii*	55	*Porphyromonas gingivalis*	95	*Rhodococcus ruber*
18	*Corynebacterium glutamicum*	56	*Prochlorococcus marinus*	96	*Klebsiella pneumoniae*
19	*Corynebacterium casei*	57	*Xanthomonas campestris*	97	*salmonella*
20	*Cyanobacteria*	58	*Pseudomonas putida*	98	*Vibrio cholerae*
21	*Vibrio parahemolyticus*	59	*Pseudomonas syringeae*	99	*Listeria monocytogenes*
22	*Dinoroseobacter shibae*	60	*Ralstonia solanacearum*	100	*Cronobacter sakazakii*
23	*Eubacterium rectale*	61	*Rhizosphere (soil bacteria)*	101	*Pseudomonas fluorescens*
24	*Erwinia amylovora*	62	*Rhodobacter sphaeroides*		
25	*Fibrobacter succinogenes*	63	*Rhodococcus*		
26	*Francisella*	64	*Roseobacter denitrificans*		
27	*Geobacter metallireducens*	65	*Salmonella typhimurium*		
28	*Hafnia alvei HA*	66	*Sodalis glossinidius*		
29	*Haemophilus ducreyi*	67	*Staphylococcus aureus*		
30	*Haloferax volcanii DS2*	68	*Streptococcus*		
31	*Helicobacter pylori*	69	*Streptomyces coelicolor*		
32	*Ideonella spp*	70	*Sulfolobus acidocaldaris*		
33	*Lactobacillus plantarum*	71	*Sulfolobus islandicus*		
34	*Lactococcus garvieae*	72	*Synechococcus*		
35	*Lactococcus lactis*	73	*Thermococcus*		
36	*Listeria monocytogenes*	74	*Themus thermophilus HB27*		
37	*Marinobacter hydrocarbonclasticus*	75	*Thermotoga maritime*		
38	*Mesotoga*	76	*Thermovibrio spp*		