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rRNA Removal (H/M/R) Kit

For human, mouse, rat RNA-seq library preparation

Cat#: AS-MB-001

Instruction Manual version 1.0

Product summary

Product description

rRNA Removal (H/M/R) Kit uses an enzymatic method to remove the highly abundant rRNAs in the total RNA for NGS RNA-seq library preparation. Both cytoplasmic (5S, 5.8S, 18S, 28S) and mitochondrial (12S and 16S) ribosomal RNAs in human, mouse, or rat total RNA samples are efficiently depleted.

Compared with mRNA enrichment by oligo(dT), rRNA Removal (H/M/R) Kit selectively digests rRNAs while retaining polyadenylated mRNAs and non-polyadenylated RNA transcripts (such as many long non-coding RNAs and antisense transcripts).

Compared with rRNA removal by oligonucleotide capture on solid phase, rRNA Removal (H/M/R) Kit is capable of removing even the fragmented rRNAs in degraded toal RNAs, which is particularly useful for FFPE RNA samples.

The rRNA-depleted RNA is conveniently purified and collected by the magnetic beads supplied in the kit.

Starting materials

- 100 ng ~ 1 µg of total RNA per reaction
- Suitable for either intact or degraded RNA (*e.g.* FFPE RNA)
- For species of human, mouse or rat

Kit components

Components	12 reactions Cat#AS-MB-001-02	Storage
Pre-treat Mix	12 µL	-20°C
Pre-treat Buffer	12 µL	-20°C
RNase Buffer	12 µL	-20°C
RNase 1	24 µL	-20°C
RNase 2	6 µL	-20°C
DNase Buffer	48 µL	-20°C
DNase	48 µL	-20°C
RNA Purification Beads	1.32 mL	4°C

Additional required materials

- Magnetic stand (1.5-mL tube compatible)
- Pipettors and pipette tips
- Thermal cycler
- Fresh 80% ethanol
- Nuclease-free water

Protocol

Part I: Pre-treatment of total RNA

1. Prepare the following Mix in a 200 µl PCR-tube per reaction:

100 ng ~ 1 µg total RNA	X µL
Nuclease-free water	Y µL
Pre-treat Mix	1 µL
Pre-treat Buffer	1 µL
Total volume	6.5 µL

2. Incubate the tube in a thermal cycler according to the temperature program:
   [95°C for 2 min; slope down to 45°C at 0.1°C/second; 45°C for 5 min].

3. Keep the tube at 45°C until the next step.

Part II: RNase digestion of rRNA

4. Prepare the following mix per reaction:

RNase Buffer	1 µL
RNase 1	2 µL
RNase 2	0.5 µL
Total volume	3.5 µL

5. Add the entire mix to the tube from Step 2. Mix thoroughly by pipetting up and down for 10 times.

6. Incubate at 45°C for 1 h and then keep at 4°C in the thermal cycler.

Part III: DNase digestion of rRNA-depleted RNA

7. Prepare the DNase digestion mix per reaction:

DNase Buffer	4 µL
DNase	4 µL
Nuclease-free water	32 µL
Total volume	40 µL

8. Add the mix to the tube from Step 6 and mix thoroughly by pipetting up and down for 10 times.

9. Incubate at 37°C for 30 minutes and then keep at 4°C in the thermal cycler.

Part IV: rRNA-depleted RNA purification

10. When the tube at step 9 reaches 4°C, transfer the 50 µL reaction solution to a nuclease-free 1.5 ml centrifuge tube.

11. Vortex the RNA Purification Beads tube to resuspend the beads.

12. Add 110 µL of the resuspended RNA Purification Beads to the 1.5 mL tube. Mix thoroughly by pipetting up and down for 10 times.

13. Incubate at room temperature for 10~15 minutes.

14. Place the tube on a magnetic stand until the supernatant becomes completely clear (about 5 minutes).

15. Carefully aspirate and discard the supernatant.

16. Keep the tube on the magnetic stand and add 200 µL of freshly prepared ethanol.

    ***Note**: Do not disturb the magnetic beads during your operation!*

17. Incubate at room temperature for 30 seconds and then discard the supernatant.

18. Repeat step 16-17 once for a total of two washes.

19. Pipette off all the residual ethanol and air dry the beads at room temperature for 5 minutes.

    ***Note**: Overly dried beads may significantly decrease the RNA recovery! If cracks are showing on the surface of the bead pellet, the beads are too dry.*

20. Take the tube off the magnetic stand and add 10~15 µL nuclease-free water.

21. Resuspend the beads by pipetting up and down for 10 times; Incubate at room temperature for 5 minutes.

22. Place the tube on the magnetic stand until the supernatant becomes completely clear (about 3 minutes).

23. Transfer the supernatant containing rRNA-depleted RNA to a new nuclease-free tube. The material is ready for NGS RNA-seq library preparation.

Troubleshooting

Problem	Possible causes	Suggestion
Too many rRNA reads in RNA-seq data	Contamination of RNase inhibitory activity in the RNA sample	Make sure no residual RNA-binding proteins or organics in your total RNA samples.
	RNase activity loss	Keep the enzymes on ice during the experiment and immediately place them back to -20°C after use.