Ribo-clean Globin mRNA Depletion Probe Mega (Prevalent Species)

RN418

Vazyme

Instruction for Use

Version 25.1

Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

Ribo-clean Globin mRNA Depletion Probe Mega (Prevalent Species) is designed to deplete globin mRNA derived from whole blood RNA of human, rat, mouse, and macaca. It is recommended to use this kit with Ribo-clean rRNA Depletion Kit Mega (Prevalent Species) (Vazyme #RN416) to deplete 5S, 5.8S, 18S, 28S rRNA, mt12S, mt16S rRNA, 45S rRNA , as well as globin mRNA (including A1/2, HBB, HBD, HBE1, HBG1/2, HBBP1, HBQ1, HBM, HBZ).

The kit is applicable to 0.01 - 1 μg of total blood RNA, and globin mRNA can be depleted through probe hybridization, RNase H digestion, and DNase I digestion. The kit is suitable for intact or partially degraded RNA samples, and the optimized reaction system improves depletion efficiency and species compatibility.

02/Target Species

Cat. No.	Application	Target Species*
RN418	Globin mRNA	Human/Rat/Mouse/*Cricetulus griseus*/ *Macaca mulatta*/*Macaca fascicularis*

* Species coverage can be found at *http://cloud.vazyme.com:83/application-tool*.

03/Components

Components	RN418-01 (6 rxns)	RN418-02 (24 rxns)	RN418-03 (96 rxns)
Globin mRNA Probe Mega (Prevalent Species)	6 μl	24 μl	96 μl

▲The color in the table corresponds to the cap color of the component.

04/Storage

Store at -30 ~ -15°C and ship at ≤0°C.

05/Applications

Ribo-clean Globin mRNA Depletion Probe Mega (Prevalent Species) is suitable for globin mRNA depletion with 0.01 - 1 μg total RNA of whole blood from human, rat, mouse, and macaca. The kit is compatible with partially degraded RNA sample, and the depleted product is applicable to RNA library preparation and other experiments. The amount of mRNA varies significantly across different samples, and the initial total RNA inputs can be adjusted according to downstream applications. For RNA library preparation, VAHTS Universal V8 RNA-seq Library Prep Kit for Illumina (Vazyme #NR605), VAHTS Universal V10 RNA-seq Library Prep Kit for Illumina (Vazyme #NR606), and VAHTS Universal V10 RNA-seq Library Prep Kit (Premixed Version) (Vazyme #NR616) are recommended.

06/Self-prepared Materials

◇ RNA Evaluation

Agilent RNA 6000 Pico Kit (Agilent #5067 - 1513).

◇ rRNA Depletion

Ribo-clean rRNA Depletion Kit Mega (Prevalent Species) (Vazyme #RN416).

◇ RNA Purification

VAHTS RNA Clean Beads (Vazyme #N412).

◇ Library Preparation Kit

VAHTS Universal V8 RNA-seq Library Prep Kit for Illumina (Vazyme #NR605),
VAHTS Universal V10 RNA-seq Library Prep Kit for Illumina (Vazyme #NR606),
VAHTS Universal V10 RNA-seq Library Prep Kit (Premixed Version) (Vazyme #NR616).

◇ Other Materials

80% ethanol (freshly prepared with Nuclease-free ddH_2O), Nuclease-free ddH_2O; Nuclease-free PCR tubes; Agilent 2100 Bioanalyzer or other equivalent products, PCR instrument, magnetic rack (Vazyme #CM101), etc.

07/Notes

07-1/About Application and Storage

1. This kit is recommended for use with Ribo-clean rRNA Depletion Kit Mega (Prevalent Species) (Vazyme #RN416).
2. To avoid repeated freeze-thaw cycles, please aliquot and store the remaining reagent after first use.

07-2/About RNA Sample Preparation

1. To ensure depletion efficiency, RNA samples should be free of ionic salts (e.g., Mg^{2+} or guanidine salts) or organic substances (e.g., phenol or ethanol), or another purification is required.
2. To avoid DNA contamination, RNA samples can be treated with DNase I to remove DNA.
3. Dilute RNA with Nuclease-free ddH_2O to 8 μl and avoid prolonged exposure on ice to prevent RNA degradation.
4. If a low RNA concentration results in an initial volume >8 μl, the RNA can be concentrated by lyophilization, ethanol precipitation, column purification or magnetic bead purification (VAHTS RNA Clean Beads, Vazyme #N412).
5. For RNA-Seq applications, the total RNA input >100 ng is recommended to increase library complexity.

07-3/About RNA Magnetic Beads

1. Equilibrate magnetic beads to room temperature and mix well before use, or the recovery efficiency may be affected.
2. Transfer the supernatant when the solution becomes clear and do not disturb the beads.

3. Use 80% ethanol (freshly prepared with Nuclease-free ddH_2O) to rinse the magnetic beads for ribosomal-depleted RNA purification; otherwise, it may cause RNA loss and library preparation failure. Please discard as much of the supernatant as possible to minimize impurity residues after second rinse.

4. Before elution, ensure that the magnetic beads are thoroughly dry (with the surface changing from shiny brown to matte brown) to prevent ethanol residue from affecting downstream experiments. However, it is important to avoid over-drying as it can reduce the RNA recovery efficiency.

07-4/About Operation

1. It is recommended to use pipette tips with filters and change the pipette tips for different samples.
2. Be sure to wear gloves during operation. Please change gloves after touching equipment or other work areas outside the RNase-free zones.
3. All reagents must be capped immediately after use to avoid contamination.
4. Briefly centrifuge enzyme components before use to avoid adhesion to the tube walls and caps.

08/Mechanism & Workflow

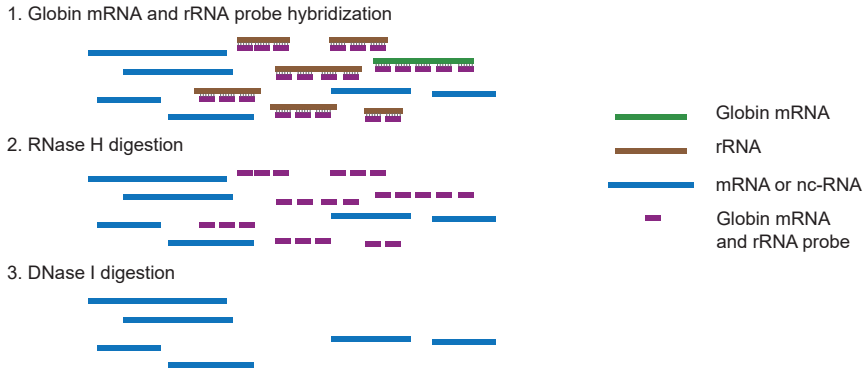

Fig 1. Schematic Diagram of Globin mRNA and rRNA Depletion

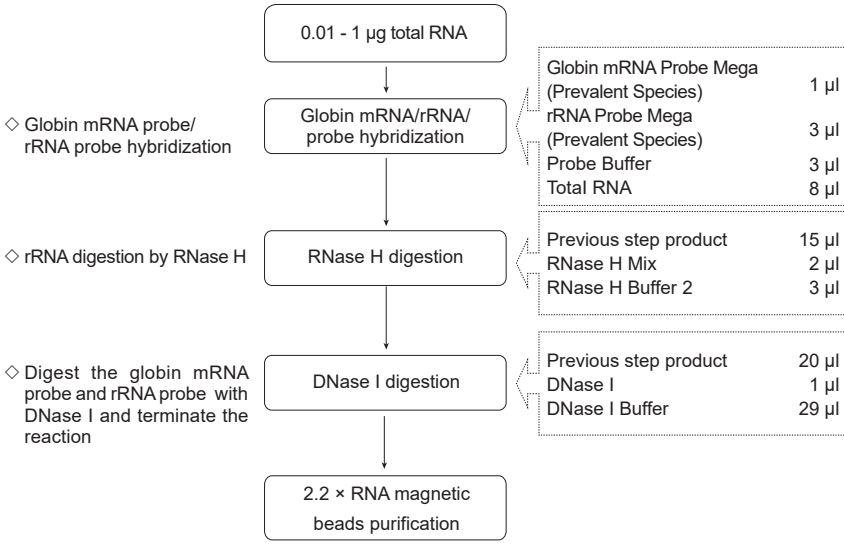

Fig 2. Workflow of Globin mRNA and rRNA Depletion

09/Experiment Process

▲ Except for Globin mRNA Probe Mega (Prevalent Species), other components are from Vazyme #RN416.

09-1/Probe Hybridization with RNA

1. Dilute 0.01 - 1 μg total RNA with Nuclease-free ddH_2O to a final volume of 8 μl in a Nuclease-free PCR tube, and keep it on ice for later use.

   ▲ Take out the required components from -30 ~ -15°C in advance, and place them on ice for later use.

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

Components	Volume
Globin mRNA Probe Mega (Prevalent Species)	1 μl
rRNA Probe Mega (Prevalent Species)	3 μl
Probe Buffer	3 μl
Total RNA	8 μl
Total	15 μl

   ▲ When multiple samples are processed simultaneously, the Globin mRNA Probe Mega, rRNA Probe Mega and Probe Buffer can be pre-mixed, and then aliquoted into each PCR tube. It is recommended to prepare 1.1 times the actual number of reactions to compensate for losses.

3. Mix well by pipetting 10 times, and briefly centrifuge to collect the reaction mix at the bottom of the tube.

4. Place the PCR tube in the PCR instrument, and perform the following program:

Temperature	Time
95°C	2 min
95 ~ 37°C	0.1°C/sec
37°C	5 min
4°C	Hold

▲ Please note that 95 ~ 37°C is a gradient annealing program.

5. Remove the tube immediately when the reaction is completed. Briefly centrifuge to collect the reaction mix at the bottom of the tube, and place it on ice for the next step.

09-2/RNase H Digestion

1. Prepare the following reaction mix on ice:

Components	Volume
RNase H Buffer 2	3 µl
RNase H Mix	2 µl
Previous step product	15 µl
Total	20 µl

2. Mix well by pipetting 10 times, and briefly centrifuge to collect the reaction mix at the bottom of the tube.

3. Place the tube in the PCR instrument, and perform the following program:

Temperature	Time
50°C	15 min
4°C	Hold

4. Briefly centrifuge to collect the reaction mix at the bottom of the tube, and place it on ice for the next step.

09-3/DNase I Digestion

1. Prepare the following reaction mix on ice:

Components	Volume
DNase I Buffer	29 µl
DNase I	1 µl
Previous step product	20 µl
Total	50 µl

2. Mix well by pipetting 10 times, and briefly centrifuge to collect the reaction mix at the bottom of the tube.

3. Place the tube in the PCR instrument, and perform the following program:

Temperature	Time
37°C	10 min
4°C	Hold

4. Briefly centrifuge to collect the reaction mix at the bottom of the tube, and place it on ice for the next step.

09-4/Ribosomal-depleted RNA Purification

1. Mix VAHTS RNA Clean Beads (Vazyme #N412) well by vortexing. Add 110 μl (2.2 ×) to the RNA sample from the previous step, and mix well by pipetting 10 times.
2. Incubate the tube on ice for 15 min to allow RNA to bind to the magnetic beads.
3. Place the tube on the magnetic rack, and discard the supernatant when the solution becomes clear (about 5 min).
4. Keep the tube on the magnetic rack, and wash the beads with 200 μl of 80% ethanol (freshly prepared with Nuclease-free ddH_2O). Incubate at room temperature for 30 sec, and then carefully discard the supernatant.
5. Repeat step 4 once.
6. Keep the tube on the magnetic rack, and air-dry the beads for 5 - 10 min.
   ▲ Do not disturb the magnetic beads when adding 80% ethanol.
   ▲ Use a 10 μl pipette to discard the residual ethanol.
   ▲ Avoid over-drying the magnetic beads, which may reduce recovery efficiency.
7. RNA elution:

Option A - Reverse transcription application:

Remove the tube from the magnetic rack, add 20 μl of Nuclease-free ddH_2O, and mix well. Incubate at room temperature for 2 min. Place the tube on the magnetic rack, and carefully transfer 18 μl of the supernatant to a new Nuclease-free PCR tube when the solution becomes clear (about 5 min). Store at -85 ~ -65°C for later use.

Option B - RNA library preparation application:

Remove the tube from the magnetic rack, add 18 μl of Frag/Prime Buffer 2 from VAHTS Universal V10 RNA-seq Library Prep Kit for Illumina (Vazyme #NR606), and mix well. Incubate at room temperature for 2 min. Place the tube on the magnetic rack, and carefully transfer 16 μl of the supernatant to a new Nuclease-free PCR tube when the solution becomes clear (about 5 min), and immediately proceed to library preparation.

10/FAQ & Troubleshooting

◇ If rRNA depletion is required for multiple species, can multiple probes be used simultaneously?

Multiple probes can be used simultaneously for rRNA depletion across various species. Follow the recommended amount of probe in the instruction manual and reduce the volume of RNA input to maintain a total volume of 15 μl.

Take multi-species rRNA depletion (Vazyme #RN416 + RN418 + RN417) for example:

Components	Volume
rRNA Probe Mega (Prevalent Species)	3 μl
Globin mRNA Probe Mega (Prevalent Species)	1 μl
rRNA Probe Mega (Bacteria)	3 μl
Probe Buffer	3 μl
Total RNA	5 μl
Total	15 μl

▲ Only the reaction system in **09-1/Probe Hybridization with RNA** is changed, while all other operations remain unchanged.

▲ If the initial amount of RNA is insufficient due to reduced volume, the RNA can be concentrated by VAHTS RNA Clean Beads (Vazyme #N412).

◇ How to know the species coverage and compatible products?

Please refer to *http://cloud.vazyme.com:83/application-tool* to get the species coverage. The species coverage is not the same as the depletion efficiency and is for reference only.

◇ How to store the purified product?

The purified product is prone to degradation due to low concentration. Proceed to the downstream experiments as soon as possible, or store at -85 ~ -65°C.

◇ If the purified product is used for library preparation, but it is eluted with Nuclease-free ddH_2O, what's the operation?

Purify the RNA again with VAHTS RNA Clean Beads (Vazyme #N412), and finally elute with Frag/Prime Buffer 2 from Vazyme #NR606.

◇ If the library concentration is too low, how to improve it?

Library concentration is related to total RNA quality including the integrity and input amounts. If it is not possible to extract qualified RNA samples, the following compensatory methods may be taken:

1. Increase total RNA inputs: up to 1 μg.
2. Prepare several duplicate samples and merge them after purification.

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