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

NB-54-0204

2x Taq Plus Master Mix (Dye Plus)

Cat# NB-54-0143-01, NB-54-0143-02

Product Description

Ribo-off Globin & rRNA Depletion Kit (Human/Mouse/Rat) is a kit for removing Globin mRNA and rRNA from total RNA in blood samples. This kit is compatible with 0.01 - 1 μg blood total RNA. The total RNA sample undergoes probe hybridization, RNase H digestion, DNase I digestion, etc. Finally, Globin mRNA and rRNA (including cytoplasmic rRNA and mitochondrial rRNA) are removed from total RNA, leaving other mRNA and non-coding RNA, which can be used for the analysis of non-coding RNA such as LncRNA. This kit is compatible with partially degraded RNA samples, and the obtained products are suitable for RNA library construction and other experiments.

Components

Components	NB-54-0204-01 (24 rxns)	NB-54-0204-02 (96 rxns)
Globin and rRNA Probe (H/M/R)	24 μl	96 μl
Probe Buffer	72 μl	288 μl
RNase H Buffer 2	72 μl	288 μl
RNase H Mix	48 μl	192 μl
DNase I Buffer	696 μl	4 × 696 μl
DNase I	24 μl	96 μl
Nuclease-free ddH₂O	1 ml	4 × 1 ml

Storage

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

Applications

Ribo-off Globin & rRNA Depletion Kit (Human/Mouse/Rat) is suitable for removing Globin mRNA (including HBA1/2, HBB, HBG1/2) and rRNA (including cytoplasmic 28S, 18S, 5.8S, 5S rRNA, and mitochondrial 16S, 12S rRNA) from 0.01 - 1 μg blood samples, leaving other mRNA and non-coding RNA. It is compatible with partially degraded RNA samples, and the products are suitable for RNA library construction and other experiments. The mRNA content in total RNA of different samples varies greatly. According to the downstream applications, the initial template amount of total RNA can be adjusted appropriately. The products can be used for library construction with VAHTS Universal V6 RNA-seq Library Prep Kit for lllumina (NeoBiotech # NB-54-0118).

Self-prepared Materials

- RNA quality control: Agilent RNA 6000 Pico Kit (Agilent #5067-1513).
- RNA purification: VAHTS RNA Clean Beads (NeoBiotech # NB-54-0061).
- Library preparation kits: VAHTS Universal V6 RNA-seq Library Prep Kit for lllumina (NeoBiotech # NB-54-0118).
- Other materials: 80% ethanol (freshly prepared with Nuclease-free ddH2O), Nuclease-free ddH2O; Nuclease-free PCR tubes, low-adsorption EP tubes (Eppendorf #022431021); Agilent 2100 Bioanalyzer or other equivalent, PCR instrument, magnetic stand, etc.

Notes

1/ About storage

1. The kit contains a variety of enzymes, which must be stored at -30 ~ -15℃. It should be kept on ice during use, otherwise the enzyme activity may be reduced.
2. In order to avoid reduced enzyme activity due to repeated freezing and thawing, it is recommended to store the remaining reagents in small aliquots after the first use.

2/ About RNA sample preparation

1. In order to ensure the removal efficiency, the RNA sample should not contain salt ions (such as Mg2+ or guanidine salt) or organic matter (such as phenol or ethanol), otherwise please perform purification again.
2. To avoid DNA contamination, RNA samples can be treated with DNase I to remove trace amounts of DNA.
3. After the RNA is diluted to 11 µl with Nuclease-free ddH2O, please do not keep it on ice for a long time to avoid RNA degradation.
4. If the initial RNA volume is >11 µl due to the low concentration, RNA can be concentrated through lyophilization, ethanol precipitation, column-based purification or magnetic beads-based purification (VAHTS RNA Clean Beads, NeoBiotech # NB-54-0061) and other methods.
5. For RNA-Seq, it is recommended that the starting amount of total RNA is higher than 100 ng to increase the complexity of the library.

3/ About magnetic beads

1. Equilibrate the beads to room temperature before use to assure capture efficiency.
2. Mix the beads thoroughly every time before pipetting.
3. The supernatant should be transferred carefully on the magnetic stand after the magnetic beads are completely adsorbed (the supernatant is clear). Avoid disturbing the magnetic beads.
4. Make sure to use freshly prepared 80% ethanol to rinse the magnetic beads, otherwise it may cause RNA loss and directly lead to the failure of library construction.
5. Do not leave any 80% ethanol supernatant behind in the second washing step to reduce the residual impurities.
6. Before elution, ensure that the magnetic beads are sufficiently dry (the surface changes from bright brown to frosted brown) to avoid residual ethanol from affecting subsequent experiments. But excessive drying (cracking) will result in loss of RNA sample.

4/ About operation

1. It is recommended to use tips with filter and change the tips between samples.
2. Please wear disposable gloves and change the glove after touching equipment outside the RNase-free areas or other working areas.
3. Close the lids of all the reagents immediately after use to avoid contamination.
4. Centrifuge the enzyme components briefly before use to avoid sticking to the tube wall and lid and causing loss.

Mechanism & Workflow

1. Globin mRNA and rRNA hybridize with probe (Probe hybridization)

2. RNase H depletion

3. DNase I depletion

Fig 1. Schematic Diagram of Globin mRNA and rRNA Removal

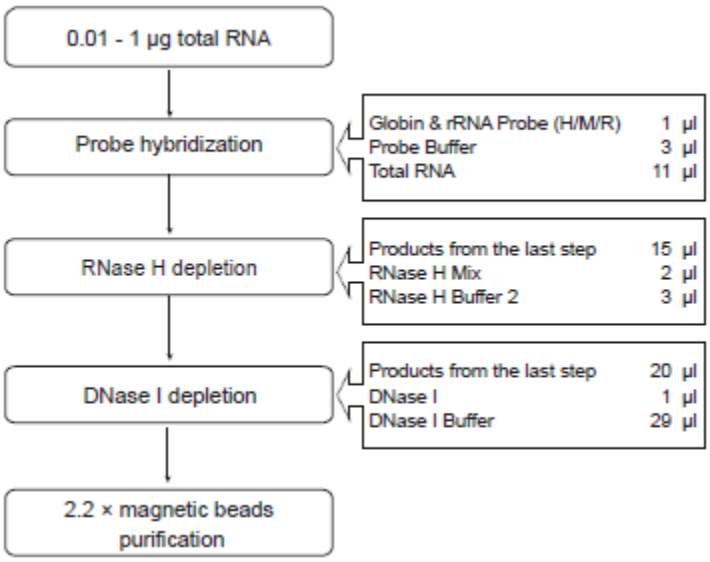

Fig 2. Operation Process of Globin mRNA and rRNA Removal

Experiment Process

1/ Probe hybridization

1. Total RNA preparation: Dilute 0.01 - 1 μg total RNA to 11 μl with Nuclease-free ddH2O in a Nuclease-free centrifuge tube and keep them on ice.

▲Thaw the required components in advance and put them on ice.

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

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

Mix thoroughly by pipetting up and down 10 times and centrifuge briefly to collect the solution at the bottom of the tubes.

▲If multiple samples are to be performed at the same time, the mixture of Globin and rRNA Probe (H/M/R) and Probe Buffer can be prepared in a centrifuge tube, and then aliquoted into each PCR tube. It is recommended to prepare the mixed solution according to 1.1 times of the actual reaction volume to make up for the loss.

3. Put the tubes into a PCR instrument and run the following program:

Temperature	Time
105°C	On
95°C	2 min
95 ~ 22°C	0.1°C/sec
22°C	5 min
4°C	Hold

▲It takes about 15 - 20 min and it may vary depend on the different types of PCR instruments.

▲Thaw the required components in advance and put them on ice.

2/ RNase H depletion

1. Prepare the following reaction mix on ice:

Components	Volume
RNase H Buffer 2	3 µl
RNase H Mix	2 µl
Products from the last step	15 µl
Total	20 µl

Mix thoroughly by pipetting up and down 10 times and centrifuge briefly to collect the solution at the bottom of the tubes.

▲If multiple samples are to be performed at the same time, the mixture of RNase H Buffer 2 and RNase H Mix can be prepared in a centrifuge tube, and then aliquoted into each PCR tube. It is recommended to prepare the mixed solution according to 1.1 times of the actual reaction volume to make up for the loss.

2. Put the tubes into a PCR instrument and run the following program:

Temperature	Time
50°C	30 min
4°C	Hold

▲Thaw the required components in advance and put them on ice.

3/ DNase I depletion

1. Prepare the following reaction mix on ice:

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

Mix thoroughly by pipetting up and down 10 times and centrifuge briefly to collect the solution at the bottom of the tubes.

▲ If multiple samples are to be performed at the same time, the mixture of DNase I Buffer and DNase I can be prepared in a centrifuge tube, and then aliquoted into each PCR tube. It is recommended to prepare the mixed solution according to 1.1 times of the actual reaction volume to make up for the loss.

2. Put the tubes into a PCR instrument and run the following program:

Temperature	Time
37℃	30 min
4℃	Hold

▲Centrifuge briefly to collect the solution at the bottom of the tubes and put them on ice. Proceed to the next step immediately.

4/ Ribosomal-depleted RNA Purification

1. Suspend the VAHTS RNA Clean Beads thoroughly by inverting or vortexing, pipet 110 µl (2.2 ×) of beads into the RNA sample of last step. Mix thoroughly by pipetting up and down 10 times.
2. Incubate on ice for 15 min to make RNA bind to the beads.
3. Place the tube on a magnetic stand. After the solution is clear (about 5 min), carefully remove and discard the supernatant.
4. Keep the tube 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.
5. Repeat step 4.
6. Keep the tube on the magnetic stand, open the lid and air-dry the beads for about 5 min.

▲Do not disturb the beads when adding 80% ethanol.

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

▲Avoid over-drying of beads, which resulting in the reduce of recovery efficiency.

7-A. If the products are used for reverse transcription, remove the tubes from the magnetic stand. Add 20 µl Nuclease-free ddH2O, pipette 10 times to mix well, and incubate at room temperature for 2 min. Place the tubes on the magnetic stand for 5 min. After the solution is clear, carefully transfer 18 µl of the supernatant into a new Nuclease-free PCR tube and store at -80 ~ -65℃ for use.

7-B. If the products are used for transcriptome library preparation, remove the tubes from the magnetic stand. Add 18.5 µl Frag/Prime Buffer, pipette 10 times to mix well, and incubate at room temperature for 2 min. Place the tubes on the magnetic stand for 5 min. After the solution is clear, carefully transfer 16 µl of the supernatant into a new Nuclease-free PCR tube and perform library preparation immediately.

9/ FAQ & Troubleshooting

How can the purified products be stored?

The purified products are easily degraded due to low concentration. Proceed to downstream experiments as soon as possible, otherwise store at -80 ~ -65℃.

If the purified products are used for library preparation, but the products are eluted with Nuclease-free ddH2O, how to do it?

When using VAHTS Universal V6 RNA-seq Library Prep Kit for Illunima (NeoBiotech #NB-54-0118), if conditions permit, add an equal volume of VAHTS 2 × Frag/Prime Buffer (NeoBiotech #NB-54-0056). Then the reaction system will be amplified until purification

step, restore the reaction system; you can also use VAHTS RNA Clean Beads (NeoBiotech #NB-54-0061) for purification again, and finally elute with Frag/Prime Buffer.

If the purified products are used for library preparation, how to choose the fragmentation conditions and the numbers of amplification cycles?

When using VAHTS Universal V6 RNA-seq Library Prep Kit for Illumina (NeoBiotech #NB-54-0118) for library preparation: The recommended fragmentation condition is 65℃ for 5 min, after performing 0.65 ×/0.1 × beads-based size selection, a library with a main peak of 350 - 450 bp can be obtained. The recommended numbers of amplification cycles are as follows:

Initial input amount	Cycles
1,000 ng	15 - 16
100 - 999 ng	18 - 19
10 - 99 ng	20 - 21

If the starting library concentration is too low, what can we do?

The yield of RNA after Globin mRNA & rRNA depletion depends on the quality of the starting RNA, the content of rRNA in the sample and the purification method used. The concentration of the library constructed with high-quality RNA samples as templates can meet the requirements of sequencing. If qualified RNA samples cannot be obtained, you can try to use the following methods to make up:

1. Initial amount: increase the initial amount of sample, the upper limit is 1 μg;
2. Make repetitions and merge them after the purification step;
3. No size selection: Although the RNA fragments are small under the fragmentation condition of 94℃ for 8 min, the distribution will be concentrated and the uniformity will be better