HB220524

Globin mRNA Depletion Probe (Human)

Product Information

Product Name	Cat#	Specification
Globin mRNA Depletion Probe (Human)	12806ES24	24 T
	12806ES96	96 T

Product Description

Globin mRNA Depletion Probe (Human) is designed to remove human Globin mRNA. It can effectively remove Globin mRNA from adult, infant and embryonic sources, including HBA1/2, HBB, HBD, HBM, HBG1/2, HBE1, HBQ1 and HBZ. This product is used with Hieff NGS™ MaxUp rRNA Depletion Kit (Human/Mouse/Rat) (Yeasen#12253) to effectively remove rRNA and Globin mRNA from total RNA. This kit is suitable for both intact and degraded RNA samples. The RNA samples after rRNA & Globin mRNA removal can be used for high-throughput sequencing analysis of mRNA and non-coding RNA, which significantly enhance the proportion of valid data in sequencing results, and cDNA synthesis or other downstream applications.

Product Application

Suitable for 100 ng~1 μg total RNA samples human, mouse and rat blood resource, and for both intact or partially degraded RNA samples.

Product Components

Product Name	Cat#	Specification
Globin Probe（human）	12806ES24	24 μl
	12806ES96	96 μl

Shipping and Storage

All the components are shipped with dry ice and can be stored at -20°C for one year.

Cautions

1. Please use consumables that are free of RNase contamination and clean the experimental area regularly. It is recommended to use ThermoFisher's RNAZap™ high-efficiency nucleic acid removal spray to remove RNase contamination.
2. The RNA sample should be free from genomic DNA contamination. If gDNA remains in the sample, it should be digested by DNase I and purified before use.
3. The maximum input volume of RNA sample is 10 μL. If the sample volume is large, it can be concentrated first.
4. For your safety and health, please wear lab coats and disposable gloves for operation.
5. For research use only!

Instructions

1. Probe Hybridization to RNA

1.1 Dilute 10 ng–1 μg of total RNA with Nuclease-free Water to a final volume of 10 μL in a PCR tube. Keep the RNA **on ice.**

1.2 Assemble the following RNA/Probe hybridization reaction **on ice** according to Table 1.

Table 1 RNA/Probe hybridization reaction

Components	Volume (μL)
Hybridization Buffer	3
Probe Mix (H/M/R)	1
Globin Probe（Human）	1
Total RNA	10 (100 ng~1 μg)
Total	15

1.3 Mix thoroughly by gently pipetting up and down at least 10 times. Briefly spin down the tube in a microcentrifuge to collect the liquid from the side of the tube.

1.4 Place tube in a thermocycler and run the following program in Table 2 with the heated lid set to 105°C.

Table 2 Reaction program of RNA/Probe hybridization

Temperature	Duration
Hot lid 105°C	On
95°C	2 min
95°C-22°C	0.1°C/s
22°C	5 min
4°C	hold

2. RNase H Digestion

2.1 Assemble the following RNase H digestion reaction **on ice** according to Table 3.

Table 3 RNase H digestion reaction

Components	Volume (μL)
RNase H Buffer	3
RNase H	2
Hybridized RNA (Step 1.4)	15
Total	20

2.2 Mix thoroughly by gently pipetting up and down at least 10 times. Briefly spin down the tube in a microcentrifuge to collect the liquid from the side of the tube.

2.3 Place tube in a thermocycler and run the following program: lid 50°C; 37°C, 30 min; 4°C, hold.

3. DNase I Digestion

3.1 Assemble the following DNase I digestion reaction on ice according to Table 4.

Table 4 DNase I digestion reaction

Components	Volume (μL)
DNase I Buffer	27.5
DNase I	2.5
RNase H treated RNA (Step 2.3)	20
Total	50

3.2 Mix thoroughly by gently pipetting up and down at least 10 times. Briefly spin down the tube in a microcentrifuge to collect the liquid from the side of the tube.

3.3 Place tube in a thermocycler and run the following program: lid off; 37°C, 30 min; 4°C, hold.

4. RNA Purification

4.1 Equilibrate the Hieff NGS^{TM}RNA Cleaner (Cat#12602) to room temperature and resuspend the beads thoroughly by vortexing before use.

4.2 Add **110 μL (2.2×)** beads to the RNA solution from Step 3.3 and mix thoroughly by pipetting up and down at least 10 times.

4.3 Incubate at room temperature for 5 minutes to bind RNA to the beads.

4.4 Place the tube on a magnetic stand to separate the beads from the supernatant. When the solution is clear (about 3 mins), discard the supernatant. Be careful not to touch the beads with the pipette tips.

4.5 Keep the tube on the magnetic stand. Add 200 μL of freshly prepared 80% ethanol to the tube. Incubate at room temperature for 30 seconds and then discard the supernatant. Be careful not to touch the beads with the pipette tips.

4.6 Repeat Step 4.5 once for a total of two washes.

4.7 Remove residual ethanol with 10 μL - pipette tips. Keep the tube on the magnetic stand and air dry-the beads for up to 5 minutes with the lid open.

4.8 Remove the tube from the magnetic stand. Elute the RNA from the beads by adding 11 μL of Nuclease-free Water. Mix thoroughly by pipetting up and down at least 10 times and briefly spin the tube.

4.9 Incubate for 5 minutes at room temperature. Place the tube on the magnetic stand until the solution is clear (~ 3 minutes).

4.10 Transfer 10 μL of the supernatant to a nuclease-free tube.

Note: If you need to stop at this point, samples can be stored at –80°C.

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