Ver. HB221111

Hieff NGS™ MaxUp Human rRNA Depletion Kit (rRNA & ITS/ETS)

Product description

Hieff NGS™ MaxUp Human rRNA Depletion Kit (rRNA & ITS/ETS) is designed to remove rRNA and 45S ITS/ETS from human, mouse and rat total RNA based on RNase H-based workflow and to retain mRNA and other non-coding RNA. This kit is suitable for both intact and partially degraded RNA samples (e.g., FFPE RNA). Since degraded FFPE samples usually contain a higher proportion of ITS/ETS than fresh tissue samples, this kit adds probes in the Human 45S ITS/ETS region, and ITS removal can significantly increase the proportion of valid data in raw data.

Components

Components No.	Name	12257ES24 (24T)	12257ES96 (96T)
12257-A	Hybridization Buffer	72 μL	288 μL
12257-B	Probe Mix (rRNA & ITS/ETS)	48 μL	192 μL
12257-C	RNase H Buffer	72 μL	288 μL
12257-D	RNase H	48 μL	192 μL
12257-E	DNase I Buffer	660 μL	2×1320 μL
12257-F	DNase I	60 μL	240 μL

Specifications

Depletion Technology	RNase H
Sample Type	Total RNA of human,mouse and rat
Final Product Type	mRNA and other non coding RNAs
No. of Reactions	24 /96 Preps
Starting Material Amount	100 ng~1 μg total RNA
Target	Remove rRNA and 45S ITS/ETS form human total RNA

Shipping and Storage

All the components are shipped with dry ice and can be stored at -15°C ~ -25°C for one year.

Instructions

Required Materials Not Included：（1）RNA clean beads: Hieff NGS™ Cleaner (Cat#12602) or equivalent；（2）qRT-PCR inspection for rRNA removal efficiency detection: Hieff™ qPCR SYBR Green Master Mix (No Rox) (Cat#11201) or equivalent；（3）Other materials: Ethanol, ddH2O, Pipettes, PCR tubes, Magnetic stand, Thermocycler.

1. Probe Hybridization to RNA

1.1 Dilute 10 ng–1 μg of total RNA with Nuclease-free Water to a final volume of 11 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
Human Probe Mix (rRNA & ITS/ETS)	2
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 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

Note: Do not premix the RNase H Buffer and RNase H.

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 50°C; 37°C, 30 min; 4°C, hold.

4. RNA Purification

4.1 Equilibrate the Hieff NGS™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.

Notes

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!