

## Hieff NGS™ MaxUp rRNA Depletion Kit (Plant)

### Product Information

Product Name	Cat#	Specification
Hieff NGS™ MaxUp rRNA Depletion Kit (Plant)	12254ES96	96 T







### Product Description

Hieff NGS™ MaxUp rRNA Depletion Kit (Plant) is designed to remove rRNA (including cytoplasmic 18S and 25S rRNA, mitochondrial 18S and 26S rRNA, chloroplast 16S and 23S rRNA) from plant 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.

### Product Application

This kit can be used for 100 ng-1 µg total RNA samples from various plants. The resulting rRNA-depleted RNA is suitable for RNA-Seq, random-primed cDNA synthesis, or other downstream RNA analysis.

### Product Components

Components	12254ES96
12254-A  Hybridization Buffer	288 µL
12254-B  Probe Mix (Plant)	288 µL
12254-C  RNase H Buffer	288 µL
12254-D  RNase H	192 µL
12254-E  DNase I Buffer	2×1320 µL
12254-F  DNase I	240 µL

### Shipping and Storage

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

### 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, ddH<sub>2</sub>O, Pipettes, PCR tubes, Magnetic stand, Thermocycler.

### 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 100 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
Probe Mix (Plant)	3
Total RNA	9 (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

- 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  $\mu\text{L}$  (2.2 $\times$ )** 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  $\mu\text{L}$  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  $\mu\text{L}$  - 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  $\mu\text{L}$  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  $\mu\text{L}$  of the supernatant to a nuclease-free tube.

*Note: If you need to stop at this point, samples can be stored at  $-80^{\circ}\text{C}$ .*

