Hieff NGS™ mRNA Isolation Master Kit

Cat# 12603

INSTRUCTION
FOR USE

Yeasen Biotechnology (Shanghai) Co., Ltd.

Table of Contents

Product Information

Product Name	Cat#	Specification
Hieff NGS™ mRNA Isolation Master Kit	12603ES24	24 T
	12603ES96	96 T

Product Description

Hieff NGS™ mRNA Isolation Master Kit is a magnetic beads kit specially developed by Yeasen Biotechnology for purification of mRNA. mRNA Capture Beads are micron grade paramagnetic microspheres coupled with Oligo (dT). The mRNA with poly (A) tails is separated and purified from total RNA with good integrity of 10 ng-4 μg.

Product Components

Components		12603ES24	12603ES96
12603-A	mRNA Capture Beads	1.2 mL	4.8 mL
12603-B	Beads Binding Buffer	1.2 mL	4.8 mL
12603-C	Beads Wash Buffer	15 mL	60 mL
12603-D	Tris Buffer	1.2 mL	4.8 mL
12603-E	Nuclease-free water	1 mL	4 mL

Shipping and Storage

All the components are shipped with ice packs and can be stored at 2-8°C for one year. Avoid freezing!

Cautions

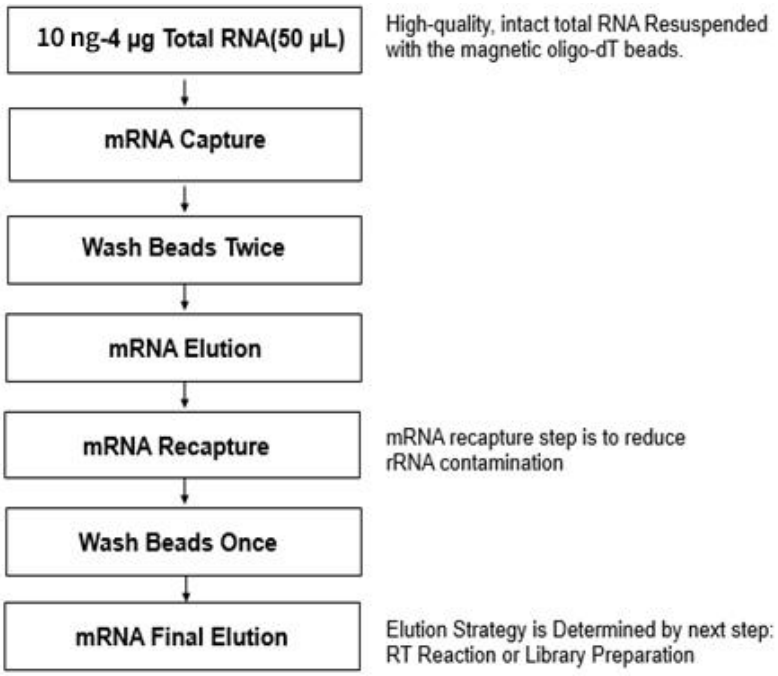
1. For your safety and health, please wear lab coats and disposable gloves for operation.
2. Thaw components at room temperature. Once the components are thawed, mix thoroughly by vortexing, spin the tube briefly and place on ice for later use.
3. Please use consumables that are free of RNase contamination and clean the experimental area regularly. It is recommended to use ThermoFisher's RNA*Zap*™ high-efficiency nucleic acid removal spray to remove RNase contamination.
4. Total RNA with good integrity and a RIN value of 7.0 or above is required for good purification.
5. For research use only!

Instructions

Part 1：Required Materials Not Included

Ethanol, ddH2O, Pipettes, PCR tubes, Magnetic stand, Thermocycler.

Part 2：Operating procedure



Picture 1 Operating procedure of mRNA purification kit

Part 3：Instructions

Step1: Preparation before experiment

Remove the mRNA Capture Beads from 2-8℃, and equilibrate to room temperature (about 30 min).

Step2：Sample preparation

Dilute 10 ng-4 μg total RNA to a final volume of 50 μL with nuclease-free water in a nuclease-free PCR tube and set on ice.

Step3：mRNA binding to magnetic beads

3.1 Invert or vortex the magnetic beads, add 50 μL magnetic beads to the RNA solution from step2, and mix thoroughly by pipetting up and down at least 10 times.

3.2 Place the tube in a thermocycler and run the following program: 65℃, 5 min; 25℃， 5 min，4℃, hold.

3.3 Place the tube on a magnetic stand for 5 min to separate the mRNA from total RNA. When the solution is clear (about 5 mins), discard the supernatant. Be careful not to touch the beads with the pipette tips.

Step 4：Sample washing

4.1 Take the tube out of the magnetic stand, add 200 μL Beads Wash Buffer and resuspend the magnetic Beads and mix thoroughly by pipetting up and down at least 6 times.

4.2 Place the tube on a magnetic stand at room temperature. When the solution is clear (about 5 mins), discard the supernatant. Be careful not to touch the beads with the pipette tips.

4.3 Repeat Step 4.1 and 4.2 once for a total of two washes.

Step 5: First round elution of the mRNA

5.1 Take the tube out of the magnetic stand, add 50 μL Tris Buffer to resuspend the magnetic Beads and mix thoroughly by pipetting up and down at least 6 times.

5.2 Place the tube in a thermocycler and run the following program: 80℃, 2 min; 25℃ hold, to elute the mRNA from the magnetic beads.

Step6：mRNA rebinding to the magnetic beads

6.1 Take the tube out of the thermocycler, add 50 μL Beads Binding Buffer and mix thoroughly by pipetting up and down.

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

6.3 Place the tube on a magnetic stand at room temperature. When the solution is clear (about 5 mins), discard the supernatant. Be careful not to touch the beads with the pipette tips.

Step7：Sample washing

7.1 Take the tube out of the magnetic holder, add 200 μL Beads Wash Buffer and resuspend the magnetic Beads and mix thoroughly by pipetting up and down at least 6 times.

7.2 Place the tube on a magnetic stand at room temperature. When the solution is clear (about 5 mins), discard the supernatant. Be careful not to touch the beads with the pipette tips.

[Note]：Use a 10 μL pipette to remove the remaining liquid.

Step8：Final elution of mRNA samples

Scheme A：For reverse transcription reaction.

A-1 Take the tube out of the magnetic holder, add 12 μL Nuclease-free water and resuspend the magnetic Beads and mix thoroughly by pipetting up and down at least 6 times.

A-2 Placed the tube in a thermocycler, hold at 80℃ for 2 min, then immediately place on the magnetic stand at room temperature for 5 min. After the solution is clear, transfer 10μL supernatant into another new nuclease-free PCR tube.

Scheme B：For RNA library preparation.

Corresponding volumes of Frag/Primer Buffer can be added according to relevant kit instructions for library preparation.

[Note]: Samples can be placed on ice for further NGS library preparation or other analytical applications (immediate follow-up is recommended) or stored at -80℃.

Memo：

BRING VALUES TO CUSTOMERS
BUILD A HEALTHIER AND HAPPIER WORLD

Yeasen Biotechnology (Shanghai) Co., Ltd.

Tel: 400-6111-883
Sales: marketing@yeasen.com
Support: marketing@yeasen.com
Service: marketing@yeasen.com
Web: www.yeabio.com