

## Hieff NGS™ mRNA Isolation Master Kit V2

### Product description

The Hieff NGS™ mRNA Isolation Master Kit V2 is an upgraded magnetic bead kit specifically designed for purifying mRNA. The mRNA Capture beads are micron-sized paramagnetic microspheres conjugated with Oligo (dT). They combine with the mRNA carrying a poly (A) tail to separate and purify the mRNA from 10 ng to 4 µg of total RNA with good integrity.

### Specifications

|         |                                               |
|---------|-----------------------------------------------|
| Cat.No. | 12629ES08 / 12629ES24 / 12629ES96 / 12629ES97 |
| Size    | 8 T / 24 T / 96 T/96 T(automated)             |

### Components

| Components No. | Name                     | 12629ES08 | 12629ES24 | 12629ES96 | 12629ES97 |
|----------------|--------------------------|-----------|-----------|-----------|-----------|
| 12629-A        | mRNA Capture Beads 2.0   | 400 µL    | 1.2 mL    | 4.8 mL    | 5.5 mL    |
| 12629-B        | Beads Binding Buffer 2.0 | 400 µL    | 1.2 mL    | 4.8 mL    | 5.5 mL    |
| 12629-C        | Beads Wash Buffer 2.0    | 5 mL      | 15 mL     | 60 mL     | 60 mL     |
| 12629-D        | Tris Buffer 2.0          | 400 µL    | 1.2 mL    | 4.8 mL    | 5.5 mL    |
| 12629-E        | Nuclease-free water      | 1 mL      | 1 mL      | 4 mL      | 4 mL      |

### Storage

This product should be stored at 2~8°C for one year.

### Instructions

#### Self-provided materials

Magnetic rack, Nuclease-free centrifuge tube, PCR instrument, etc.

#### 1.1 Preparation before the Experiment

Take the mRNA Capture Beads 2.0 out from the storage condition at 2~-8°C and let it stand still to equilibrate to room temperature (about 30 minutes).

#### 1.2 Sample Preparation

In a nuclease-free PCR tube, dilute 10 ng - 4 µg of total RNA to a volume of 50 µL with nuclease-free water. Place it on ice for standby.

#### 1.3 Binding of mRNA to Magnetic Beads

(1) Invert or vortex the magnetic beads to mix them evenly. Aspirate **50 µL of mRNA Capture Beads 2.0** and add it to the 50 µL total RNA sample. Pipette up and down 6 times to ensure thorough mixing.

(2) Place the mixture of magnetic beads and RNA in a PCR instrument and set the following conditions: **65°C for 5 minutes; 25°C for 5 minutes; then hold at 25°C** to complete the binding of RNA to the capture magnetic beads.

(3) Place the sample on a magnetic rack and let it stand still at room temperature for 5 minutes to separate the mRNA from the total RNA. Carefully remove the supernatant.

#### 1.4 Washing of the Sample

(1) Take the sample out from the magnetic rack, add **200 µL of Beads Wash Buffer 2.0** to resuspend the magnetic beads, and pipette up and down 6 times to mix thoroughly.

(2) Place the sample on the magnetic rack and let it stand still at room temperature for 5 minutes. Carefully remove the supernatant.

(3) Repeat the above step for a total of two washes.

#### 1.5 First-round Elution of the mRNA Sample

(1) Take the sample out from the magnetic rack, add **50 µL of Tris Buffer 2.0** to resuspend the magnetic beads, and pipette up and down 6 times to mix thoroughly.

(2) Place the sample in a PCR instrument and set the conditions as follows: **80°C for 2 minutes and then hold at 25°C** to elute the mRNA.

#### 1.6 Re-binding of mRNA to Magnetic Beads

(1) Take the sample out from the PCR instrument, add **50 µL of Beads Binding Buffer 2.0**, and pipette up and down 6 times to mix thoroughly.

(2) Incubate at room temperature for 5 minutes to allow the mRNA to bind to the magnetic beads.

(3) Place the sample on the magnetic rack and let it stand still at room temperature for 5 minutes. Carefully remove the supernatant.

#### 1.7 Washing of the Sample

Take the sample out from the magnetic rack, add **200 µL of Beads Wash Buffer 2.0** to resuspend the magnetic beads, pipette up and down 6 times to mix thoroughly, then place the sample back on the magnetic rack, let it stand still at room temperature for 5 minutes, and then aspirate all the supernatant.

**[Note]:** Finally, use a 10 µL pipette to aspirate the residual liquid completely.

#### Select the treatment method according to the subsequent experimental process:

a. If the purified product is used for reverse transcription reaction, take the sample out from the magnetic rack, add 10.5 µL of nuclease-free water, pipette up and down 6 times to mix thoroughly, heat it at 80 °C for 2 minutes, immediately place it on the magnetic rack for 5 minutes. After the solution becomes clear, carefully aspirate 8.5 µL of the supernatant into a new nuclease-free PCR tube.

b. If the purified product is used for RNA library construction, such as the Hieff NGS™ Ultima Dual-mode RNA Library Prep Kit (compatible with Illumina and MGI) (Cat #12308 or 12310), add the corresponding volume of Frag/Prime Buffer according to the instruction manual and conduct library construction.

#### Notes

1. Before using magnetic beads, it is essential to allow them to warm back to room temperature and mix them thoroughly. Otherwise, it may affect the recovery efficiency of samples.

2. During the operation process, it is necessary to strictly ensure that there is RNase free and nucleic acid contamination.

3. When this product is used in combination with other reagents, please operate according to the specific experimental instructions.
4. To obtain a good purification effect, it is required to have total RNA with good integrity and ensure that the RIN (RNA Integrity Number) value is 7.0 or above.
5. For your safety and health, please wear a laboratory coat and disposable gloves when operating.
6. This product is only for research !