MagicPure® mRNA Kit

Please read the manual carefully before use.
Cat. No. EC511
Version No. Version 4.0
Storage: at 2-8℃ for one year

Description

MagicPure® mRNA Kit uses oligo(dT)-conjugated magnetic beads to specifically bind to poly(A) tailed mRNA. It is suitable for isolating mRNA from purified highly intact total RNA (0.01-10 μg, RIN value≥8). The isolated mRNA can be used in RT-PCR, qRT-PCR, next generation sequencing, or other applications. This kit is compatible with magnetic-rod high-throughput nucleic acid extractor.

Highlights

• High-yield and high-purity isolated mRNA
• Simple workflow

Sample Requirements

0.01-10 μg of purified highly intact total RNA (RIN value≥8)

Kit Contents

Component	EC511-01 (24 rxns)	EC511-02 (96 rxns)
Binding Buffer 33 II (BB33 II)	1.3 ml	5 ml
Clean Buffer 33 (CB33)	1.3 ml	5 ml
Wash Buffer 33 II (WB33 II)	10 ml	40 ml
RNase-free Water	1.3 ml	5 ml
mRNA Beads II	1.3 ml	5 ml

Procedures

1. Take mRNA Beads out from 2-8℃ and equilibrate to room temperature for 30 minutes. Mix well by vortexing.
2. Prepare RNA sample: dilute the total RNA to 50 μl with RNase-free Water in a PCR tube.
3. Pipette 50 μl of Beads to the RNA sample. Mix well by pipetting up and down.
4. Place the PCR tube in a PCR machine at 65°C for 5 minutes, 25°C for 5 minutes, and hold at 4°C to allow the mRNA to bind to the magnetic beads.
   Note: Make sure that the beads have been mixed thoroughly prior to reaction.
5. Place the PCR tube on a magnetic stand for 5 minutes. Discard the supernatant carefully and completely.
6. Remove the PCR tube from the magnetic stand. Add 200 μl of WB33 II and mix well by pipetting. Place on the magnetic stand for 5 minutes and discard the supernatant carefully and completely.
7. Remove the PCR tube from the magnetic stand. Add 50 μl of CB33 and resuspend the beads by pipetting.
8. Heat the tube for 2 minutes at 80℃ and cool to 25℃.
9. Add 50 μl of BB33 II and mix well by pipetting. Incubate at room temperature for 5 minutes.
10. Place the PCR tube on the magnetic stand for 5 minutes. Discard the supernatant carefully and completely.
11. Remove the PCR tube from the magnetic stand. Add 200 μl of BB33 II and mix well by pipetting. Place on the magnetic stand for 5 minutes and discard the supernatant carefully and completely.
12. Choose the processing method according to the experimental process:
    a. Use automated instrument is used for purification or the purified product is used for reverse transcription: Remove the tube from the magnetic stand. Add 18.5 μl RNase-free Water. Pipet 6 times to mix thoroughly. Incubate at 80°C for 2 minutes. Immediately place the tube on the magnetic stand for 5 minutes. After the solution is clear, carefully pipet 17 μl of the supernatant to into a new RNase-free PCR tube.
    b. Purified product is used for RNA library construction, such as TransNGS Fast RNA Seq Library Prep Kit for Illumina Rapid RNA Library Prep Kit (Cat. No. KP701): Add 1×RNA Fragmentation Buffer according to the manual for library construction.
13. The purified product can be placed on ice for NGS library construction or other analysis applications (it is recommended to carry out subsequent reactions immediately), or it can be stored at -80°C.

Notes

• Please use RNase-free PCR tubes;

• The total RNA sample should be highly intact (RIN value>8). Otherwise the mRNA information will be partially lost.

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