Library Preparation VAHTS™ mRNA Capture Beads

N401-01/02

Version 8.1

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Introduction

The Library Preparation VAHTS™ mRNA Capture Beads is 1 μm paramagnetic beads coupling of Oligo d (T) which is applicable to isolate intact poly $(A)^+$ RNA from previously isolated total RNA. Magnetic separation technology permits elution of intact mRNA in small volumes eliminating the need for precipitating the poly $(A)^+$ transcripts in the eluent. The entire operation can be completed in less than one hour.

Components

Components	N401-01 (24 rxn)	N401-02 (96 rxn)
mRNA Capture Beads	1.2 ml	4.8 ml
Beads Binding Buffer	1.2 ml	4.8 ml
Beads Wash Buffer	9.6 ml	38.4 ml
Tris Buffer	1.2 ml	4.8 ml
Nuclease-free Water	1 ml	4 ml

Storage Conditions

Store at 4℃.

Other Materials Required

Magnetic Stand

Nuclease-free PCR tube

Application

Isolation of poly$(A)^+$ RNA transcript from 0.5 μg - 11 μg of intact Total RNA, Incomplete or degraded total RNA template will result in information missing of partial poly$(A)^+$ RNA.

Protocol

1. Equilibrate mRNA Capture Beads to room temperature.
2. Prepare the RNA sample: Dilute the total RNA with nuclease-free water to a final volume of 50 μl in a nuclease-free PCR tube and place it on ice for later use.
3. Mix the mRNA Capture Beads thoroughly by inverting or vortexing, pipette 50 μl of the mRNA Capture Beads into the total RNA sample. Pipette up and down slowly 6 times to mix thoroughly.
4. Place the sample in the PCR instrument, 65℃ for 5 min, and 4℃ hold, to denature the RNA.
5. Place the tube at room temperature for 5 minutes to allow the mRNA to bind to the magnetic beads.
6. Place the sample on the magnetic stand for 5 minutes to separate the mRNA from the total RNA; carefully remove the supernatant
7. Remove the sample from the magnetic stand, mix thoroughly with 200 μl Beads Wash Buffer by pipetting 6 times, stand on the magnetic stand for 5 minutes, and carefully remove the supernatant.
8. Remove the sample from the magnetic stand and resuspend the beads with 50 μl Tris Buffer; Pipette up and down slowly 6 times to mix thoroughly.
9. Place the sample in the PCR instrument, 80℃ for 2 min, hold at 25℃, to elute the mRNA.
10. Add 50 μl of Beads Binding Buffer and pipette up and down slowly 6 times to mix thoroughly.
11. Leave at room temperature for 5 minutes to allow the mRNA to bind to the magnetic beads.

12. Place the sample on the magnetic stand for 5 minutes to separate the mRNA from the total RNA; carefully remove the supernatant.
13. Remove the sample from the magnetic stand, mix thoroughly with 200 μl Beads Wash Buffer by pipetting 6 times, place on the magnetic stand for 5 minutes, and carefully remove all supernatant.

14A. If the purified product is used for reverse transcription reaction, remove the sample from the magnetic stand, add 10.5 μl Nuclease-free water, pipette up and down slowly 6 times to mix thoroughly, 80℃ for 2 min, and immediately placed on a magnetic stand for 5 minutes. Carefully pipette 8 μl of the supernatant into a new Nuclease-free PCR tube after the solution was clarified.

14B. If the purified product is used in RNA library preparation, please refer to the manual of VAHTS™ mRNA-seq v2 Library Prep Kit for Illumina® Kit (Vazyme #NR601) or VAHTS™ Stranded mRNA-seq Library Prep Kit for Illumina® Kit (Vazyme #NR602) to added appropriate volume of Frag/Prime Buffer for library construction.

15. Place the sample on ice for further NGS library preparation or other analytical applications (recommended to immediately proceed to the following reactions) or store it at -20℃.