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Seq-Star™ poly(A) mRNA Isolation Kit

-for NGS RNA-Seq library preparation

Cat#: AS-MB-006

Instruction Manual version 1.0

Product summary

Product description

Seq-Star™ poly(A) mRNA Isolation kit is designed to isolate highly pure and intact poly(A) mRNA from total RNA samples ideal for NGS RNA-seq library preparation. The poly(A) mRNA is enriched by oligo(dT) conjugated magnetic beads. The kit components and procedure are highly optimized for quality and convenience. Each reaction can purify 1~5 µg total RNA. The purified poly(A) mRNA is eluted in a small volume and ready for use, without the need for precipitation.

Starting materials

- 1~5 µg of high quality total RNA in nuclease-free water per reaction. Partially degraded total RNA may lose 5' portions of the isolated mRNAs

Kit components

Components	24-Reactions Cat#AS-MB-006-01	96-Reactions Cat#AS-MB-006-02	Storage
Seq-Star™ oligo(dT) Beads	480 µL	1.92 mL	4°C
RNA Binding Buffer	8 mL	30 mL	4°C
Wash Buffer	30 mL	120 mL	4°C
Elution Buffer	6 mL	24 mL	4°C
User Manual	√	√	

Additional required materials

- Magnetic stand (tube compatible)
- Pipettors and tips
- Heat block or thermo cycler
- Nuclease-free water

Protocol

Part I: oligo(dT) beads preparation

1. Aliquot 20 µL of Seq-Star™ oligo(dT) Beads per sample in nuclease-free tubes.
2. Put the tube on Magnetic stand until the supernatant becomes completely clear (about 2 minutes).
3. Carefully aspirate and discard the supernatant.
4. Take the tube off the magnetic stand and add 100 µL RNA Binding Buffer. Mix thoroughly by pipetting up and down 10 times.
5. Put the tubes on the magnetic stand until the supernatant becomes completely clear. Remove and discard the supernatant.
6. Repeat Step 4-5 once for a total of two washes.
7. Resuspend the beads in 50 µL RNA Binding Buffer. Keep at room temperature.

Part II: First round of mRNA binding

8. Add nuclease-free water to the total RNA solution for a final total volume of 50 µL.
9. Transfer 50 µL oligo(dT) beads from Step 7 to the total RNA sample. Mix thoroughly.
10. Incubate the mix at 65°C for 5 minutes and cool to 4°C in a thermal cycler.
11. Take the tube from the thermal cycler when the temperature reaches 4°C.
12. Pipette up and down slowly 10 times to resuspend the beads. Incubate at room temperature for 5 minutes.
13. Resuspend the beads by pipetting up and down 10 times. Incubate again at room temperature for 5 minutes.
14. Place the tube on a magnetic stand until the supernatant becomes completely clear (about 2 minutes).
15. Carefully aspirate and discard the supernatant. Take the tube off the magnetic stand.
16. Add 200 µL Wash Buffer and resuspend the beads.
17. Place the tube on magnetic stand until the supernatant becomes completely clear (about 2 minutes). Carefully aspirate and discard all the supernatant.
18. Repeat Step 16-17 once for a total of two washes.

19. Add 50 µL of Elution Buffer to the beads and gently pipette up and down 10 times to mix thoroughly.
20. Incubate the tube at 80°C for 2 minutes and then hold at 25°C on a thermal cycler.

Part III: Second round of mRNA binding

21. Take the tube from the thermal cycler when the temperature reaches 25°C.
22. Add 50 µL of RNA Binding Buffer to the tube and gently pipette up and down 10 times to mix thoroughly.
23. Incubate the tube at room temperature for 5 minutes.
24. Resuspend the beads by pipetting up and down 10 times. Incubate again at room temperature for 5 minutes.
25. Place the tube on magnetic stand until the supernatant becomes completely clear (about 2 minutes).
26. Carefully aspirate and discard the supernatant. Take the tube off the magnetic stand.
27. Add 200 µL Wash Buffer and resuspend the beads.
28. Place the tube on the magnetic stand until the supernatant becomes completely clear (about 2 minutes).
29. Carefully aspirate and discard all the supernatant.

Part IV: Elution of poly(A) mRNA

30. Take the tube off the magnetic stand. Add 15~17 µL Elution Buffer to resuspend the beads by pipetting up and down 10 times.
31. Incubate the tube at 80°C for 2 minutes and then immediately place the tube on the magnetic stand until the supernatant becomes completely clear (about 2 minutes).
32. Transfer 14~16 µL supernatant to a new nuclease-free tube.
33. The supernatant contains the purified poly(A) mRNA, which is ready for RNA-seq library preparation or other use.

Troubleshooting

Problem	Possible causes	Suggestion
Low yields	Degraded staring total RNA.	Check the integrity of the staring total RNA. High quality, undegraded intact total RNA is recommended.
	Magnetic bead loss during the process	Take care not to pipette off any oligo(dT) beads during the process.
Problems in RNA-seq library preparation	Salt carryover	Make sure to remove all residual supernatant in the tube after each wash step!