7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com
Email: info@abpbio.com

NGS Poly(A) mRNA Isolation Module

Catalog Number: E116-1, E116-2

Table 1. Kit Components and Storage

Kit Component	E116-1 (25 rxn)	E116-2 (100 rxn)	Storage	Stability
MagPure Oligo(dT)$_{25}$ Beads	1.25 mL	5.0 mL	4 °C	The product is stable for 12 months when stored as directed.
Binding Buffer (2×)	6.5 mL	26 mL		
Wash Buffer	20 mL	80 mL		
Nuclease-free Water	3 mL	12 mL		

Product Description

The NGS Poly(A) mRNA Isolation Module is designed to isolate intact poly(A) mRNA from previously isolated total RNA. The technology is based on the coupling of Oligo d(T)$_{25}$ to paramagnetic beads which is then used as the solid support for the direct binding of poly(A) mRNA. Thus, the procedure permits the manual processing of multiple samples and can be adapted for automated high-throughput applications. Additionally, magnetic separation technology permits elution of intact mRNA in small volumes eliminating the need for precipitating the poly(A)+ transcripts in the eluent.

Applications

- Isolation of poly(A)+ RNA transcript from total RNA for RNA library preparation and sequencing.

Protocol

1. Dilute the total RNA (1-5 µg) with nuclease-free water to a final volume of 50 µL in a nuclease-free 0.2 mL PCR tube.
2. In a second nuclease-free 0.2 mL PCR tube aliquot 50 µL of well resuspended MagPure Oligo d(T)$_{25}$ Beads.
3. Wash the beads by adding 100 µL of Binding Buffer to the beads. Pipette the entire volume up and down 6 times to mix thoroughly.
4. Place the tube on the magnetic rack at room temperature for 2 minutes or until the solution is clear.
5. Remove and discard all of the supernatant from the tube. Take care not to disturb the beads.
6. Remove the tube from the magnetic rack.
7. Repeat steps 3-6 once for a total of 2 washes.
8. Resuspend the beads in 50 µL of Binding Buffer and add the 50 µL of total RNA sample from step 1. Pipette the entire volume up and down to mix thoroughly.
9. Place the tubes on the thermal cycler and heat the sample at 65°C for 2 minutes and hold at 20°C to denature the RNA and facilitate binding of the poly(A) RNA to the beads.
10. Remove tubes from the thermal cycler when the temperature reaches 20°C.
11. Resuspend the beads. Pipette up and down slowly 6 times to mix thoroughly.
12. Place the tubes on the bench and incubate at room temperature for 5 minutes to allow the RNA to bind to the beads.
13. Resuspend the beads. Pipette up and down slowly 6 times to mix thoroughly.

14. Incubate for 5 more minutes on the bench at room temperature to allow the RNA to bind to the beads.
15. Place the tubes on the magnetic rack at room temperature for 2 minutes or until the solution is clear to separate the poly(A) RNA bound to the beads from the solution.
16. Remove and discard all of the supernatant. Take care not to disturb the beads.
17. Remove the tubes from the magnetic rack.
18. Wash the beads by adding 200 µL of Wash Buffer to remove unbound RNA. Pipette the entire volume up and down 6 times to mix thoroughly.
19. Place the tubes on the magnetic rack at room temperature for 2 minutes or until the solution is clear.
20. Remove and discard all the supernatant. Take care not to disturb the beads.
21. Remove the tubes from the magnetic rack.
22. Repeat Steps 18-21.
23. Add 50 µL of nuclease-free water to each tube. Gently pipette the entire volume up and down 6 times to mix thoroughly.
24. Place the tubes on the thermal cycler. Close the lid and heat the sample at 70°C for 2 minutes, then hold at 20°C to elute the poly(A) RNA from the beads.
25. Remove the tubes from the thermal cycler when the temperature reaches 20°C.
26. Add 50 µL of Binding Buffer to each sample to allow the RNA to bind to the same beads. Gently pipette the entire volume up and down 6 times to mix thoroughly.
27. Incubate the tubes on the bench at room temperature for 5 minutes.
28. Resuspend the beads. Pipette up and down slowly 6 times to mix thoroughly.
29. Incubate the tubes on the bench at room temperature for 5 more minutes to allow the RNA to bind to the beads.
30. Place the tubes on the magnetic stand at room temperature for 2 minutes or until the solution is clear.
31. Remove and discard all of the supernatant from each tube. Take care not to disturb the beads.
32. Remove the tubes from the magnetic rack.
33. Wash the beads once with 200 µL of Wash Buffer. Gently pipette the entire volume up and down 6 times to mix thoroughly.
34. Place the tubes on the magnetic rack at room temperature for 2 minutes or until the solution is clear.
35. Remove and discard all of the supernatant from each tube. Take care not to disturb the beads.
36. Remove the tubes from the magnetic rack.
37. If elution is required, add 10-20 µL of nuclease-free water. Heat to 70°C for 2 minutes and place the tube immediately on the magnet. Transfer the eluted mRNA to a new RNase-free tube.