# Isolate mRNA using the NEBNext® Poly(A) mRNA Magnetic Isolation Module (E7490)

## Overview

**Note:** If this module is being used with either NEBNext Ultra™ Directional RNA Library Prep Kit for Illumina (NEB #E7420) or NEBNext Ultra RNA Library Prep Kit for Illumina (NEB #E7530) or NEBNext RNA First Strand Synthesis Module (NEB #E7525) **do not follow the protocol below.** The correct protocol can be found in NEB #E7420, NEB #E7525 or NEB #E7530 manuals.

**Note:** For best results keep all the reagents used during the Poly(A) isolation except the NEBNext Oligo $d(T)_{25}$ beads, **on ice** when not in use.

**Starting Material:** 1–5 µg of DNA-free total RNA.

1. Dilute the total RNA 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 20 µl of well resuspended NEBNext Magnetic Oligo $d(T)_{25}$ Beads.
3. Wash the beads by adding 100 µl of RNA 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 RNA 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 5 minutes and hold at 4°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 4°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 from each well of the plate. 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 Tris Buffer 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 80°C for 2 minutes, then hold at 25°C to elute the poly-A RNA from the beads.

25. Remove the tubes from the thermal cycler when the temperature reaches 25°C.

26. Add 50 µl of RNA 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.

    **Note: It is important to remove all of the supernatant to successfully use the RNA in downstream steps. Spin down the tube. Place the tube on the magnetic rack and with a 10 µl tip remove all of the wash buffer. (Caution: Do not disturb beads that contain the mRNA).**

36. Remove the tubes from the magnetic rack.

37. Elute the mRNA from the beads by adding 17 µl of the Tris Buffer, mix by pipetting 6 times and incubating the sample at 80°C for 2 minutes, then hold at 25°C to elute the polyA RNA from the beads. Immediately, place the tubes on the magnetic rack for 2 minutes or until the solution is clear.

38. Collect the purified mRNA by transferring the supernatant to a clean nuclease-free PCR Tube.

39. Place tube on ice.

40. Assess the Yield and the Size Distribution of the purified mRNA. Run 1 µl on the Bioanalyzer using a RNA Pico Chip. You may have to dilute your sample before loading.