LIBRARY PREPARATION

NEBNext® Poly(A) mRNA Magnetic Isolation Module

Instruction Manual

NEB #E7490S/L
24/96 reactions
Version 6.0   4/18

be INSPIRED
drive DISCOVERY
stay GENUINE

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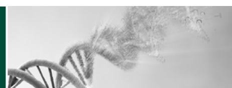

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The NEBNext Poly(A) mRNA Magnetic Isolation Module Includes:

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7490S) and 96reactions (NEB #E7490L). All reagents should be stored at 4°C.

NEBNext Oligo $d(T)_{25}$ Beads

NEBNext RNA Binding Buffer (2X)

NEBNext Wash Buffer

NEBNext Tris Buffer

Nuclease-free Water

Required Materials Not Included:

96-well 0.2 ml PCR Plates and Microseal® 'B' Adhesive Sealer (BioRad MSB-1001) or 0.2 ml RNase-free tube.

Magnetic Rack (Alpaqua, cat #A001322 or equivalent)

Thermal cycler or heat block

Bioanalyzer® (Agilent Technologies, Inc.) or similar instrument and consumables

Description:

The NEBNext Poly(A) mRNA Magnetic Isolation Module is designed to isolate intact poly(A)+ RNA from previously isolated total RNA. The technology is based on the coupling of Oligo $d(T)_{25}$ to 1 µm paramagnetic beads which is then used as the solid support for the direct binding of poly(A)+ RNA. 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. Intact poly(A)+ RNA which is fully representative of the mRNA population of the original sample can be obtained in less than one hour.

Application:

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

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

Note: If this module is being used with either NEBNext Ultra™ Directional RNA Library Prep Kit for Illumina (NEB #E7420), NEBNext Ultra RNA Library Prep Kit for Illumina (NEB #E7530), NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760), NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads (NEB #E7765), NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770), NEBNext Ultra II RNA Library Prep with Sample Purification Beads (NEB #E7775), NEBNext RNA First Strand Synthesis Module (NEB #E7525) or NEBNext Ultra II RNA First Strand Synthesis Module (NEB #E7771) **do not follow the protocol below**. The correct protocol can be found in NEB #E7420, #E7530, #E7760, #E7765, #E7770, #E7775, #E7525 or #E7771 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 tube. 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.

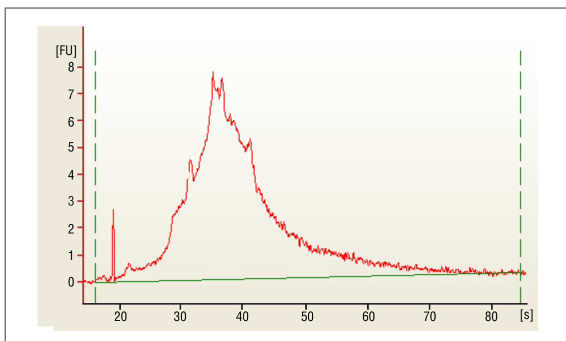
37. Elute the mRNA from the beads by adding 17 µl of the Tris Buffer, mix by pipetting 6 times and incubating the sample at 80°C for 2 minutes, then hold at 25°C to elute the polyA RNA from the beads. Immediately, place the tubes on the magnetic rack for 2 minutes or until the solution is clear.

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.

Figure 1: Example of mRNA distribution on a bioanalyzer.



Checklist:

[_] 1. Dilute RNA to 50 µl in 0.2 ml tube

[_] 2. New 0.2 ml tube: aliquot 20 µl NEBNext Magnetic Oligo $d(T)_{25}$ Beads

[_] 3. Add 100 µl of RNA Binding Buffer and mix 6 times

[_] 4. Place tube on magnet for 2 minutes

[_] 5. Remove and discard supernatant

[_] 6. Remove tube from magnet

[_] 7. Repeat Steps 3 [_], 4 [_], 5 [_] and 6 [_]

[_] 8. Resuspend beads in 50 µl RNA Binding Buffer, add 50 µl total RNA sample

[_] 9. Heat the sample at 65°C for 5 minutes, then hold at 4°C

[_] 10. Remove tubes when temperature is 4°C

[_] 11. Resuspend beads by mixing slowly 6 times

[_] 12. Incubate tubes on bench for 5 minutes

[_] 13. Resuspend beads by mixing slowly 6 times

[_] 14. Incubate tubes 5 more minutes on bench

[_] 15. Place tubes on magnet for 2 minutes

[_] 16. Remove and discard supernatant

[_] 17. Remove tube from magnet

[_] 18. Wash beads with 200 µl Wash Buffer by mixing 6 times

[_] 19. Place tubes on magnet for 2 minutes

[_] 20. Remove and discard supernatant

[_] 21. Remove tubes from magnet

[_] 22. Repeat Steps 18 [_], 19 [_],20 [_] and 21 [_]

[_] 23. Add 50 µl Tris to tubes and mix 6 times

[_] 24. Place tubes on thermal cycler, heat at 80°C for 2 minutes, then hold at 25°C

[_] 25. Remove tubes from thermal cycler when temperature is 25°C

[_] 26. Add 50 µl RNA Binding Buffer to same beads in tubes and mix 6 times

[_] 27. Incubate tubes at room temperature for 5 minutes

[_] 28. Resuspend beads by mixing slowly 6 times

[_] 29. Incubate tubes on bench for 5 more minutes

[_] 30. Place tubes on magnet for 2 minutes

[_] 31. Remove and discard supernatant

[_] 32. Remove tubes from magnet

[_] 33. Wash beads with 200 µl of Wash Buffer by mixing 6 times

[_] 34. Place tubes on magnet for 2 minutes

[_] 35. Remove and discard supernatant

[_] 36. Remove tubes from magnet

[_] 37. Add 17 µl Tris Buffer to elute mRNA, incubate at 80°C for 2 minutes, immediately put on magnet

[_] 38. Transfer RNA in supernatant to a new tube

[_] 39. Put tube on ice

[_] 40. Run mRNA on Bioanalyer Pico Chip

Kit Components

NEB #E7490S Table of Components

NEB #	PRODUCT	VOLUME
E7499A	NEBNext Oligo $d(T)_{25}$ Beads	0.480 ml
E7492A	NEBNext RNA Binding Buffer (2X)	7.2 ml
E7493A	NEBNext Wash Buffer	28.8 ml
E7496A	NEBNext Tris Buffer	6.0 ml
E7495A	Nuclease-free Water	1.2 ml

NEB #E7490L Table of Components

NEB #	PRODUCT	VOLUME
E7499AA	NEBNext Oligo $d(T)_{25}$ Beads	1.92 ml
E7492AA	NEBNext RNA Binding Buffer (2X)	28.8 ml
E7493AA	NEBNext Wash Buffer	57.6 ml
E7496AA	NEBNext Tris Buffer	24.0 ml
E7495AA	Nuclease-free Water	4.8 ml

Revision History:

REVISION #	DESCRIPTION	DATE
1.2	Added protocol warning note to page 3. Renamed NEBNext Elution Buffer to NEBNext Tris Buffer.	
2.0	Volume of beads increased from 15 µl to 20 µl. Additional mixing and incubation steps were added after each thermocycler incubation.	7/15
3.0	Update the protocol "Isolate mRNA using the NEBNext Oligo d(T)25 Magnetic Beads."	3/16
4.0	Adding clarification to many steps, broke out steps into more individual steps. Added to keep reagents on ice. Adjusted the title of the protocol.	5/16
5.0	Component volume change E7493AA.	11/16
5.1	Added note to protocol.	7/17
6.0	Create "Kit Component – Table of Components" for small and large size kits. Delete individual component information pages.	4/18

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