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NEBNext® Poly(A) mRNA Magnetic Isolation Module NEB #E7490S/L (Standard Protocol)

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Abstract

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. The new protocol for poly(A) mRNA enrichment (Section 1, Express Protocol) enables comparable performance to our current protocol (Section 2, Standard Protocol) using a substantially faster and more streamlined workflow.

Guidelines

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Application

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

Materials

The NEBNext Poly(A) mRNA Magnetic Isolation Module Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7490S) and 96 reactions (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 ([M] 10 millimolar (mM) Tris HCl, pH 7.5)
- Nuclease-free Water

Required Materials Not Included

- 96-well 0.2 ml PCR Plates and Microseal® 'B' Adhesive Sealer (Bio-Rad® MSB-1001) or 0.2 ml RNase-free tube, for example Tempassure
- PCR flex-free 8-tube strips (USA Scientific® #1402-4708)
- Magnetic Rack (NEB#S1515S, Alpaqua®, cat. #A001322 or equivalent)
- 1.5 ml Microcentrifuge tube and NEB #S1506 Magnet stand or equivalent (for washing beads only)
- Thermal cycler or heat block
- Bioanalyzer® (Agilent® Technologies, Inc.) or similar instrument and consumables

Before start

Note: When using NEBNext Poly(A) mRNA Magnetic Isolation Module E7490 with NEBNext library preparation kits, follow the protocol in the respective library prep kit manual or see Appendix A. Modifications to elution buffer and volumes will need to be made at Step 40 to make the enriched mRNA directly compatible with the different library preparation workflows.

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.

The protocol has been optimized using high quality Universal Human Reference Total RNA.

RNA Integrity

Assess the quality of the Input RNA by running the RNA sample on an Agilent Bioanalyzer RNA 6000 Nano/Pico Chip. For Poly(A) mRNA enrichment, high quality RNA with a RIN score > 7 is required.

RNA Sample Requirements

The RNA sample should be free of salts (e.g., Mg^{2+}, or guanidinium salts, divalent cation chelating agents (e.g., EDTA or EGTA) or organics (e.g., phenol or ethanol). RNA must be free of DNA. gDNA is a common contaminant from RNA preps. It may be carried over from the interphase of organic extractions or when the silica matrix of solid phase RNA purification methods is overloaded. If the total RNA sample may contain gDNA contamination, treat the sample with DNase I to remove all traces of DNA (DNase is not provided in this kit). After treatment with DNase I the enzyme should be removed from the sample. Any residual activity of the DNase I may degrade the oligos necessary for the enrichment. DNase I can be removed from the extraction using phenol/chloroform extraction and ethanol precipitation.

Starting Material: 1–5 µg* of DNA-free total RNA (Standard Protocol)

35m

1

Note

*Inputs as low as 10 ng can be used in combination with NEBNext library preparation workflows. Please refer to the respective library prep manual for the appropriate instructions to follow. Modifications to elution buffer and volumes will need to be made here to make the enriched mRNA directly compatible with the different NEBNext library preparation workflows. Please refer to Appendix A and consult the appropriate manual for more information.

Dilute the total RNA with nuclease-free water to a final volume of 50 µL in a nuclease-free 0.2 ml PCR tube and keep On ice .

2 To wash the NEBNext Oligo d(T)25 Beads, add the following to a 1.5 ml nuclease-free tube. If preparing multiple libraries, beads for up to 10 samples can be added to a single 1.5 ml tube for subsequent washes (use magnet NEB #S1506 for 1.5 ml tubes). The purpose of this step is to bring the beads from the storage buffer into the binding buffer. The NEBNext RNA Binding Buffer (2X) does not have to be diluted for this step.

	A	B
	COMPONENT	VOLUME PER ONE LIBRARY
	NEBNext Oligo dT Beads d(T)25	20 µl
	NEBNext RNA Binding Buffer (2X)	100 µl
	Total Volume	120 µl

3 Wash the beads by pipetting up and down 6 times.

4 Place the tube on the magnetic rack at Room temperature for 00:02:00 or until the solution is clear.

2m

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 Add 100 µL NEBNext RNA Binding Buffer (2X) to the beads and wash by pipetting up and down 6 times. If preparing multiple libraries, add 100 µL RNA Binding Buffer per sample. The Binding Buffer does not have to be diluted.

8 Place the tubes on the magnet and incubate at Room temperature until the solution is clear (~ 2 minutes).

9 Remove and discard the supernatant from the tube. Take care not to disturb the beads.

10 Remove tubes from magnet and add 50 µL NEBNext RNA Binding Buffer (2X) to the beads and mix by pipetting up and down until beads are homogenous. If preparing multiple libraries, add 50 µL RNA Binding Buffer per sample. The Binding Buffer does not have to be diluted.

11 Add 50 µL beads to each RNA sample from Step 1. Mix thoroughly by pipetting up and down 6 times. This first binding step removes most of the non target RNA.

12 Place the tubes on the thermal cycler and heat the sample at 65 °C for 00:05:00 and **cool to** 4 °C **with the heated lid set at ≥** 75 °C to denature the RNA and facilitate binding of the mRNA to the beads.

5m

13 Remove tubes from the thermal cycler when the temperature reaches 4 °C .

14 Resuspend the beads. Pipette up and down slowly 6 times to mix thoroughly.

15 Place the tubes on the bench and incubate at Room temperature for 00:05:00 to allow the RNA to bind to the beads.

5m

16 Resuspend the beads. Pipette up and down slowly 6 times to mix thoroughly.

17 Incubate for 5 more minutes on the bench at Room temperature to allow the RNA to bind to the beads.

18 Place the tubes on the magnetic rack at Room temperature for 00:02:00 or until the solution is clear to separate the poly(A) RNA bound to the beads from the solution. 2m

19 Remove and discard all of the supernatant. Take care not to disturb the beads.

20 Remove the tubes from the magnetic rack.

21 Wash the beads by adding 200 µL of NEBNext Wash Buffer to remove unbound RNA. Pipette the entire volume up and down 6 times to mix thoroughly.

22 Place the tubes on the magnetic rack at Room temperature for 00:02:00 or until the solution is clear. 2m

23 Remove and discard all the supernatant from each well of the tube. Take care not to disturb the beads.

24 Remove the tubes from the magnetic rack.

25 Repeat Steps 21–24

26 Add 50 µL of NEBNext Tris Buffer (provided in NEB #E7490 kit) to each tube. Gently pipette the entire volume up and down 6 times to mix thoroughly.

27 Place the tubes on the thermal cycler. Close the lid and heat the sample at 80 °C for 00:02:00, then cool to 25 °C with the heated lid set at ≥ 90 °C to do the first elution of the mRNA from the beads. 2m

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

29 Add 50 µL of NEBNext RNA Binding Buffer (2X) to each sample to allow the RNA to re-bind to the same beads. The Binding Buffer does not have to be diluted. Gently pipette the entire volume up and down 6 times to mix thoroughly.

30 Incubate the tubes on the bench at Room temperature for 00:05:00 . 5m

31 Resuspend the beads. Pipette up and down slowly 6 times to mix thoroughly.

32 Incubate the tubes on the bench at Room temperature for 5 more minutes to allow the RNA to bind to the beads.

33 Place the tubes on the magnetic stand at Room temperature for 00:02:00 or until the solution is clear. 2m

34 Remove and discard all of the supernatant from each tube. Take care not to disturb the beads.

35 Remove the tubes from the magnetic rack.

36 Wash the beads once with 200 µL of NEBNext Wash Buffer. Gently pipette the entire volume up and down 6 times to mix thoroughly. Spin down the tube briefly to collect the liquid from the wall and lid of the tube.

37 Place the tubes on the magnetic rack at Room temperature for 00:02:00 or until the solution is clear. 2m

38 Remove and discard all of the supernatant from each tube. Take care not to disturb the beads.

Note

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 NEBNext Wash Buffer. Caution: Do not disturb beads that contain the mRNA. Avoid letting the beads dry out before adding elution buffer.

39 Remove the tubes from the magnetic rack.

Note

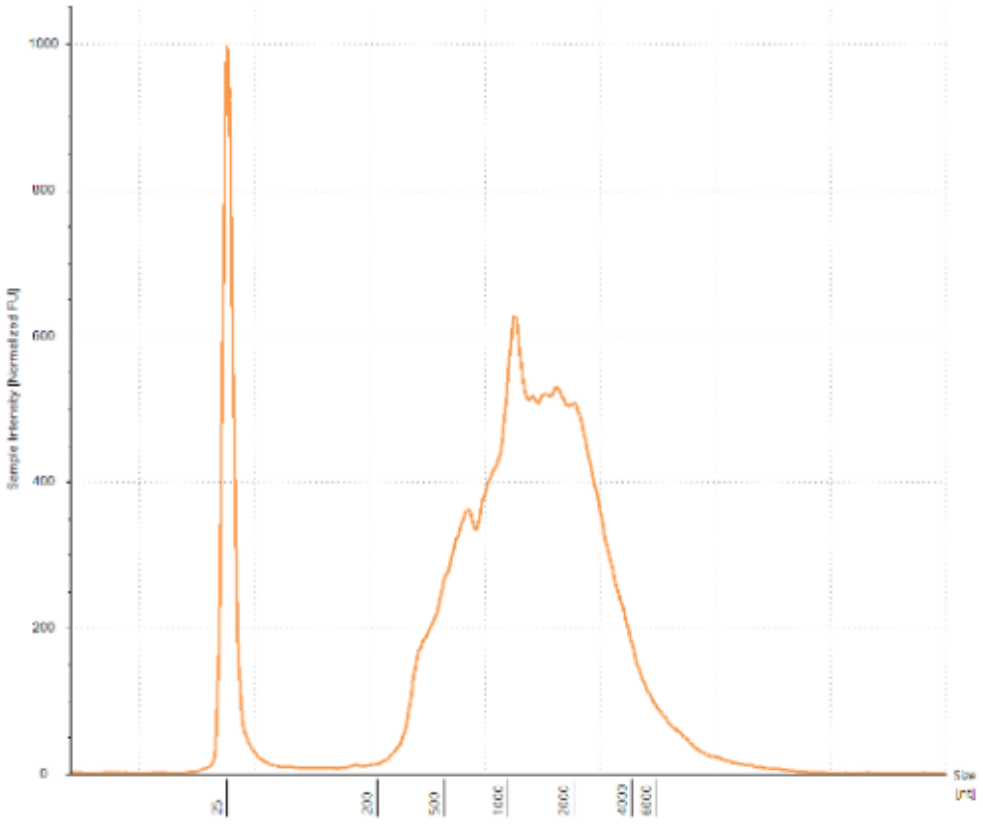
Note: Modifications to elution buffer and volumes will need to be made here to make the enriched mRNA directly compatible with the different NEBNext library preparation workflows. Please refer to Appendix A and consult the appropriate manual for more information.

40 Elute the mRNA from the beads by adding 17 µL of the NEBNext Tris Buffer, mix by pipetting 6 times and incubate the samples at 80 °C for 00:02:00, then cool to 25 °C. Immediately, place the tubes on the magnetic rack for 00:02:00 or until the solution is clear.

4m

41 Collect the purified mRNA by transferring 15 µL of the supernatant to a clean nuclease-free PCR Tube. Place On ice if using immediately. Alternatively, the sample can be placed at -80 °C for long term storage.

42 Assess the Yield and the Size Distribution of the purified mRNA. Run 1 µL on the Bioanalyzer using a RNA Pico Chip or the Tapestation using a High Sensitivity RNA ScreenTape. You may have to dilute your sample before loading.



Example of mRNA distribution on a TapeStation.