Magnetic mRNA Isolation Kit

NEB #S1550S

25 isolations

Version 4.0_4/25

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The Magnetic mRNA Isolation Kit Includes:

The Magnetic mRNA Isolation Kit contains all reagents necessary to perform 25 isolations at the largest recommended scale.

Oligo d(T)$_{25}$ Magnetic Beads (5 mg/ml)

Lysis/Binding Buffer

Wash Buffer I

Wash Buffer II

Low Salt Buffer

Elution Buffer

Required Materials Not Included:

RNase-free microcentrifuge tubes, pipette tips and pipettors

Magnetic Separation Rack (i.e., NEB #S1506 or #S1507)

Rotary or end-over-end type agitator

Sterile 21-gauge needle and syringe

Introduction

The New England Biolabs Magnetic mRNA Isolation Kit is designed to isolate intact poly(A)$^+$ RNA from crude cell lysates or tissue without requiring phenol or other chaotropic reagents. The technology is based on the coupling of oligo d(T)$_{25}$ to 1 µm superparamagnetic beads which are then used as the solid support for the direct binding of poly(A)$^+$ RNA. 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 precipitation. Other advantages are: (1) Isolation of intact poly(A)$^+$ RNA, which is fully representative of the mRNA population of the original sample in less than one hour. (2) The beads can be reused up to three times from the same input source without regeneration. (3) The covalently bound d(T)$_{25}$ DNA may be used as a primer during first-strand cDNA synthesis.

Isolated poly(A)$^+$ RNA is usually greater than 70% pure. Ribosomal RNA (rRNA) and trace amounts of genomic DNA (gDNA) are sometimes seen as impurities. The presence of rRNA will not interfere with the analysis of results from Northern Blotting and RT-PCR

experiments. Trace gDNA contamination is usually low enough to permit valid qRT-PCR quantitation of mRNA transcripts without further purification.

Isolation Preparation

Allow all kit components to come to room temperature.

Resuspend Oligo d(T)$_{25}$ beads by agitating at room temperature (RT) for 30 minutes.

Reuse of Oligo d(T)$_{25}$ beads for second-round of poly(A)$^+$ selection

Oligo d(T)$_{25}$ beads can be re-used for a second-round of purification of a poly(A)$^+$ RNA eluent without regeneration. After the elution of mRNA from the beads, wash beads once with an additional 100 µl of pre-warmed (50ºC) elution buffer. Place in magnetic rack and pull beads to the side of the tube. Remove and discard wash solution. Wash beads once with 200 µl of Lysis/Binding Buffer then re-apply previously isolated eluent to beads after adjusting salt concentration to 0.5 M LiCl or NaCl. Repeat isolation steps.

Regeneration of Oligo d(T)$_{25}$ beads

Oligo d(T)$_{25}$ beads can be regenerated and used to isolate RNA from a different source. Add 0.1 N NaOH to the beads and incubate at room temperature with agitation for 5 minutes. Wash the beads three times with elution buffer (or sterile RNAse-free H_2O), equilibrate the beads by washing three times with sterile RNase-free 1X PBS (pH 7.4) containing 0.1% Tween 20 and store in sterile RNase-free 1X PBS (pH 7.4) containing 0.1% Tween 20 and 0.02% NaN_3.

Isolation of mRNA from Mammalian Cells

Precautions should be taken to avoid ribonuclease contamination during the isolation procedure. Clean the area where isolation will be performed with RNase decontamination reagent before starting the isolation.

Wear latex gloves or equivalent at all times when handling kit components.

Step 1

Aliquot the appropriate volume of Oligo d(T)$_{25}$ beads for the scale of isolation (Table 1).

Add 200 µl of Lysis/Binding Buffer to beads, vortex briefly and mix with agitation for 2 minutes. Beads should remain in the lysis/binding solution until removal immediately before adding the cell lysate.

Step 2

Adherent cells

1. Aspirate media from cell culture plate and wash once with cold sterile 1X PBS (pH 7.4).
2. Add the appropriate volume of Lysis/Binding Buffer (Table 1) to cells and gently swirl by hand.

Suspension cells

1. Pellet cells by centrifuging at 1,000 rpm for 5 minutes at 4°C.
2. Aspirate media and wash once with cold sterile 1X PBS (pH 7.4).
3. Pellet again, discard PBS and add the appropriate volume of Lysis/ Binding Buffer (Table 1) to cells.
4. Agitate to suspend cells in Lysis/Binding Buffer.

Step 3

Incubate at RT for 5 minutes with gentle agitation. The viscosity of the solution will increase, indicating effective lysis is achieved. If the solution is too viscous (which can lead to bead clumping and gDNA carryover), pass the lysate several times through a 21-gauge needle (avoid foaming) attached to a 1 or 2-ml syringe. A noticeable decrease in viscosity should be observed.

Place the microcentrifuge tube containing the beads and lysis/binding wash into the magnetic rack and pull the magnetic beads to the side of the tube.

Step 4

Remove Lysis/Binding buffer from the beads and add the cell lysate to the equilibrated magnetic beads.

Pipette mix to fully re-suspend bead pellet then place cell lysate-and-bead suspension on the agitator and mix at RT for 10 minutes.

Place microcentrifuge tube into the magnetic rack and pull magnetic beads to the side of the tube, remove and discard supernatant.

Step 5

Add the appropriate volume of Wash Buffer 1 (Table 1) to the beads and pipette mix to fully re-suspend bead pellet, incubate with agitation for 1 minute.

Place microcentrifuge tube into magnetic rack and pull magnetic beads to the side of the tube, remove and discard wash solution.

Repeat once.

Step 6

Add the appropriate volume of Wash Buffer 2 (Table 1) to the beads and pipette mix to fully re-suspend bead pellet, incubate with agitation for 1 minute.

Place microcentrifuge tube into the magnetic rack and pull magnetic beads to the side of the tube, remove and discard wash solution.

Repeat once.

Step 7

Add the appropriate volume of Low Salt Buffer (Table 1) to the beads and pipette mix to fully re-suspend bead pellet, incubate with agitation for 1 minute.

Place microcentrifuge tube in the magnetic rack and pull magnetic beads to the side of the tube, remove and discard wash solution.

Step 8

Add the appropriate volume of Elution Buffer (Table 1) and vortex gently to suspend beads.

Incubate at 50°C for 2 minutes with occasional agitation to elute poly(A)$^+$ RNA. Greater than 90% of the poly(A)$^+$ RNA bound to the beads is recovered in this step.

Place microcentrifuge tube in the magnetic rack and pull the magnetic beads to the side of the tube. Transfer eluent to a clean, sterile RNase-free tube and store on ice for immediate quantitation or place at –80°C for long-term storage.

Quantitation of Isolated Poly(A)$^+$ RNA

Refer to page 5.

Figure 1. Lysis and binding.

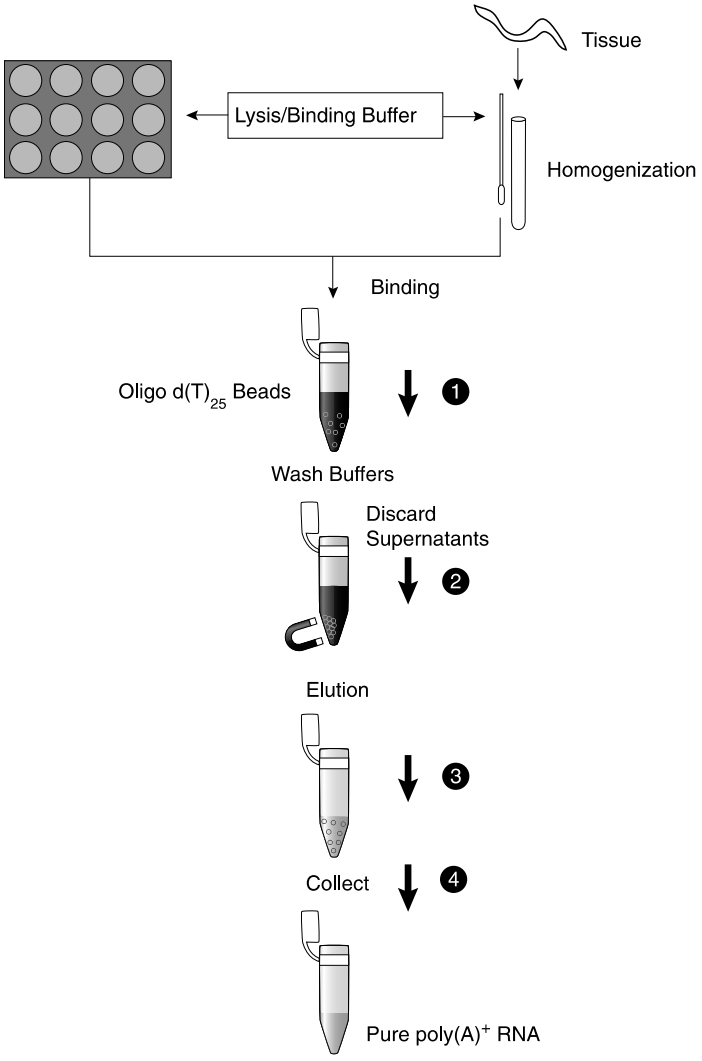

Table 1. Recommended volumes for different isolation scales (for cells).

NUMBER OF CELLS	VOLUME OF OLIGO $D(T)_{23}$ BEADS	VOLUME OF LYSIS/BINDING BUFFER	VOLUME OF WASH BUFFERS & LOW SALT BUFFER	VOLUME OF ELUTION BUFFER	EXPECTED YIELD (µg)
5 x 10^4	50 µl	250 µl	250 µl	50 µl	~0.5–2
1 x 10^5	100 µl	500 µl	500 µl	100 µl	~1–3
5 x 10^5	100 µl	500 µl	500 µl	100 µl	~2–4

Isolation of mRNA from Tissue

Precautions should be taken to avoid ribonuclease contamination during isolation of the RNA. All materials used during the isolation procedure should be sterile and RNase-free.

Clean the homogenizer with a NaOH based detergent and rinse with sterile RNase-free deionized H_2O.

Wear latex gloves or equivalent at all times when handling kit components.

Reserve reagents exclusively for RNA work. If possible separate procedures such as plasmid preps which require the use of RNases from this work area.

Increasing the amount of tissue sample beyond the recommended amount at a given scale does not increase the yield of poly(A)$^+$ RNA isolated. More importantly, this will cause an increase the amount of tissue debris and ribonucleases present during the isolation.

Step 1

Aliquot the appropriate volume of Oligo $d(T)_{25}$ beads for the scale of isolation (Table 2).

Add 200 µl of Lysis/Binding Buffer to beads, vortex briefly and mix with agitation for 2 minutes. Beads should remain in the lysis/binding wash solution until removal immediately before adding the cell lysate.

If tissue has been prepared beforehand and frozen, add Lysis/Binding directly to frozen tissue sample and proceed to Step 4, below.

Step 2

Weigh fresh or frozen animal tissue as quickly as possible to avoid mRNA degradation.

Place tissue into liquid nitrogen and grind immediately.

Step 3

Transfer ground tissue to a new tube containing the appropriate volume of Lysis/Binding Buffer (Table 2) and homogenize using brief 20-second pulses.

If the solution is viscous, pass the lysate several times through a 21-gauge needle attached to a 1 or 2 ml syringe. A noticeable decrease in viscosity should be observed.

Step 4

Spin sheared lysate at 12,000 rpm, 4°C for 1 minute.

Place the microcentrifuge tube containing the beads and lysis/binding wash into the magnetic rack and pull the magnetic beads to the side of the tube, remove and discard the wash solution.

Step 5

Decant tissue supernatant and add to previously washed Oligo $d(T)_{25}$ beads. Pipette mix to fully re-suspend bead pellet, place lysate-and-bead suspension on the agitator and incubate at RT for 10 minutes.

Place microcentrifuge tube into the magnetic rack and pull magnetic beads to the side of the tube, remove and discard supernatant.

Step 6

Add the appropriate volume of Wash Buffer 1 (Table 2) to the beads, pipette mix to fully re-suspend bead pellet and incubate with agitation for 1 minute. Place microcentrifuge tube in the magnetic rack and pull magnetic beads to the side of the tube, remove and discard wash solution.

Repeat once.

Step 7

Add the appropriate amount of Wash Buffer 2 (Table 2) to the beads. Pipette mix to fully re-suspend bead pellet and incubate with agitation for 1 minute.

Place microcentrifuge tube in the magnetic rack and pull magnetic beads to the side of the tube, remove and discard wash solution.

Repeat once.

Step 8

Add the appropriate volume of Low Salt Buffer (Table 2) to the beads. Pipette mix to fully re-suspend bead pellet and incubate with agitation for 1 minute.

Place tube in magnetic rack and pull magnetic beads to the side of the tube, remove and discard wash solution.

Step 9

Add 100 µl of Elution Buffer and vortex gently to suspend beads.

Incubate at 50°C for 2 minutes with occasional agitation to elute poly(A)$^+$ RNA. Greater than 90% of the poly(A)$^+$ RNA bound to the beads is recovered in this step.

Place microcentrifuge tube in magnetic rack and pull magnetic beads to the side of the tube.

Transfer eluent to a clean, sterile RNase-free tube. Store on ice and immediately quantitate or place at –80°C for long-term storage.

Table 2. Recommended isolation scale (for tissue).

RNA SOURCE	TISSUE (MG) PER ISOLATION	VOLUME OF OLIGO d(T)$_{25}$ BEADS	VOLUME OF LYSIS/BINDING BUFFER	VOLUME OF WASH BUFFERS & LOW SALT BUFFER
Animal Tissue	10 mg	100 µl	500 µl	500 µl
	20 mg	200 µl	500 µl	500 µl

Quantitation of Isolated Poly(A)$^+$ RNA

At this point quantification of isolated poly(A)$^+$ RNA can be done by measurement of the A_{260} absorbance using a Nanodrop™ spectrophotometer to avoid dilution of the sample. The $A_{260/280}$ ratio should be 1.6 or greater.

The amount of isolated RNA will vary with the source of the RNA sample, typically for RNA 1 A_{260} Unit = ~ 40 µg /ml.

The eluted mRNA should be placed in the magnetic rack while removing aliquots for quantitation to avoid pipetting of beads, which will interfere with spectrophotometric analysis. The instrument should be blanked with the appropriate elution buffer.

Troubleshooting

Viscous lysate

Certain cells lines and types of tissue will produce lysates that are more viscous than normal. Pass these cell or tissue lysates several times through a 21-gauge needle. If sample lysate remains viscous after additional shearing, proceed with isolation. Poly(A)$^+$ RNA can be isolated under these conditions but is more likely to be contaminated with gDNA.

Clumping of Beads

If lysate- bead- suspension clumps, rapidly pipette up and down with a 1 ml pipetman to further reduce viscosity. Dilution with additional Lysis/Binding buffer will not reverse the clumping. Proceed with isolation, poly(A)$^+$ RNA of > 60% purity can be isolated under these conditions.

Genomic DNA Contamination

Lower the ratio of cells or tissue to Lysis/Binding Buffer volume. Increase the binding incubation time if the lysate remains viscous after additional shearing. If intended downstream application requires mRNA be free of gDNA, a second round of isolation should be done. See reuse of Oligo d(T)$_{25}$ Magnetic Beads page 2.

Low Yield of mRNA

Increase the ratio of beads to Lysis/Binding Buffer or add RNase-free Proteinase K to the sample lysate directly before the 10 minute binding incubation.

References

1. Wu, N. et al. (2004) *Molecular Biotechnology*, 27, 119–126.

Appendix

Buffer Composition

Lysis/Binding Buffer:	100 mM Tris-HCl, pH 7.5, 500 mM LiCl, 0.5% LiDS, 1 mM EDTA, 5 mM DTT
Wash Buffer I:	20 mM Tris-HCl, pH 7.5, 500 mM LiCl, 0.1% LiDS, 1 mM EDTA, 5 mM DTT
Wash Buffer II:	20 mM Tris-HCl, pH 7.5, 500 mM LiCl, 1 mM EDTA
Low-Salt Buffer:	20 mM Tris-HCl, pH 7.5, 200 mM LiCl, 1 mM EDTA
Elution Buffer:	20 mM Tris-HCl, pH 7.5, 1 mM EDTA

Ordering Information

NEB #	PRODUCT	SIZE
S1550S	Magnetic mRNA Isolation Kit	25 isolations

KIT COMPONENTS SOLD SEPARATELY

NEB #	PRODUCT	SIZE
S1419S	Oligo d(T)$_{25}$ Magnetic Beads	10 µg

COMPANION PRODUCTS

NEB #	PRODUCT	SIZE
S1506S	6-Tube Magnetic Separation Rack	6 tubes
S1509S	12-Tube Magnetic Separation Rack	12 tubes

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	
1.1		6/11
2.0		8/18
3.0	Apply new manual format.	8/20
4.0	Update protocol and update formatting and legal text	4/25