Quick mRNA Purification Kit

Affinity purification is a versatile and particular technique for purifying all classes of biomolecules by taking advantage of differences in the biological activities of chemical structures—this technique's excellent selectivity results in good purification and high recovery.

BcMag™ Quick mRNA Purification Kit is designed to isolate intact poly(A)+ RNA from cells and tissue without the use of phenol or other chemical solvents. The method works by attaching Oligo $d(T)_{25}$ to 1µm paramagnetic beads, which are subsequently used as a firm platform for direct binding of poly(A)+ RNA. As a result, the approach allows for the manual processing of several samples while still being adaptable for automated high-throughput applications. Furthermore, magnetic separation technology allows for the elution of intact mRNA in small amounts, avoiding the need for the poly(A)+ transcripts to precipitate in the eluent. In less than an hour, intact poly(A)+ RNA fully representative of the original sample's mRNA population can be produced**.**

Workflow

The purification with magnetic beads is straightforward (Fig.1). Mix the beads with the cell lysates and incubate them with continuous rotation for a sufficient time. The beads remain suspended in the sample solution during mixing, allowing the mRNA to bind to oligo-dT magnetic beads. Ribosomal RNA and tiny RNA molecules (transfer RNA, microRNA, small nucleolar RNA, and small cytoplasmic RNA) do not adhere to the beads and are eliminated. After incubation, only polyadenylated RNA species (mRNA) are collected and separated from the sample using a magnet rack. Then the ultrapure mRNAs are eluted and used in downstream applications

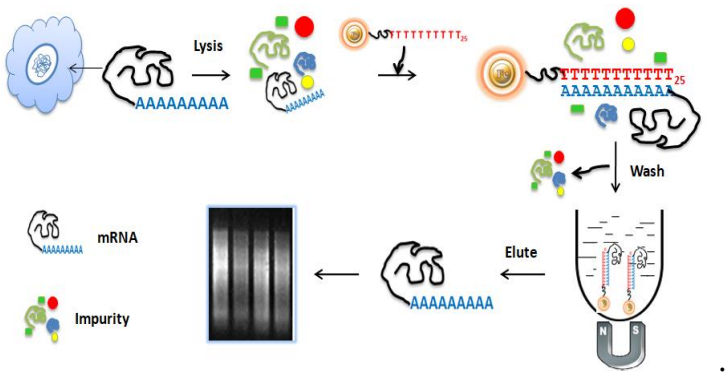

Fig.1 Workflow for quick mRNA purification

Features and Advantages

- Suitable for high-throughput automated applications
- No phenol or other organic solvents are required.
- Precipitation of poly(A)+ transcripts in eluent is not required.
- Genomic DNA contamination is minimal.
- Beads that are reusable
- Get pure mRNA in less than an hour.
- Save time. Directly isolate mRNA from cell lysates and tissue homogenates (isolation of total RNA not required).
- Prepare mRNA suitable for practically every downstream application.
- Save time. Isolate mRNA directly from cell lysates and tissue homogenates (isolation of total RNA not required).
- Allow for a diverse set of samples. Use both small and large-scale mRNA preparations.

Applications:

- cDNA synthesis
- cDNA library creation
- RT-PCR, quantitative RT-PCR
- RPA (Ribonuclease Protection Assay)
- Subtractive hybridization • Dot/slot hybridization

Product Specificities	
Composition	Magnetic beads linked with oligo-dT (25)
Bead Size	~1µm diameter
Number of Beads	~1.7 x 10^8 beads (1µm beads) /mg
Stability	pH -6-10
Magnetization	~40-45 EMU/g
Type of Magnetization	Superparamagnetic
Effective Density	2.5 g/ml
Concentration	10 mg/ml in TE Buffer
Binding Capacity	10 µg mRNA / ml of beads
Storage	Store Magnetic beads at 4°C, Buffers at room temperature upon receipt

Products

Components	Storage	Cat #: MMS-101	Cat #: MMS-102
BcMag™ Oligo-dT Magnetic beads	4°C	2 ml	4 ml
1x Binding/ Lysis Buffer	4°C	10 ml	
10x Washing Buffer I	4°C	5ml	
10x Washing Buffer II	4°C	5 ml	
Elution Buffer	4°C	0.5 ml	

Protocol

Note:

- Different samples contain a variable amount of total RNA. At the same time, only 1-5% of total RNA is mRNA.
- Each user must estimate the yield of RNA from the experimental sample. If the yield of RNA is much lower than expected, the following causes should be considered.

Materials Required

Buffer:

- BcMag oligo-d$(T)_{25}$ magnetic beads: 10 mg/ml in 50 mM Tris-HC, pH 7.5, 0.5 M NaCl, 1mM EDTA, 0.1% NaN_3
- 1x mRNA Binding Buffer: 0.1 M HEPES, pH 7.5, 0.5 M LiCl, 10 mM EDTA, 1% SDS, 10mM Dithiothreitol (DTT)
- Washing Buffer I: 10mM HEPES, pH 7.5, 0.15 M LiCl, 1mM ETDA, 0.1% SDS
- Washing Buffer II: 10mM HEPES, pH 7.5, 0.15 M LiCl, 1mM ETDA
- Elution Buffer: 10mM HEPES, pH 7.5

Equipment

- Magnetic Rack (for manual operation)

  Based on sample volume, the user can choose one of the following magnetic Racks: BcMag Rack-2 for holding two individual 1.5 ml centrifuge tubes (Cat. # MS-01); BcMag Rack-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag Rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag Rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Cat. # MS-04); BcMag™ Rack-96 for holding a 96 ELISA plate or PCR plate (Cat. # MS-05). For larger scale purification, Ceramic magnets Block for large scale purification (6 in x 4 in x 1 in block ferrite magnet, Applied Magnets, Cat# CERAMIC-B8)
- Corning 430825 cell culture flask for large-scale purification (Cole-Parmer, Cat#EW-01936-22)

- Mini BlotBoy 3D Rocker, fixed speed, small 10" x 7.5" platform w/ flat mat (Benchmark Scientific, Inc. Cat# B3D1008) or compatible

  Note: Contaminating RNAse or improper handling quickly degrades RNA molecules during purification. For the best results, follow the following guidelines.

  - Try to use fresh samples. Samples should be quickly frozen in liquid nitrogen and stored at –80ºC.
  - All glassware and plastic containers should be incubated in 1.0 M NaOH for four h to eliminate RNAse,
  - and then thoroughly rinsed with ultrapure water. However, disposable plastic tubes or other containers that have been pre-sterilized can be used directly.
  - . Fresh ultrapure water (Milli-Q grade) can directly be used to prepare solutions.
  - Always wear disposable gloves and change them frequently.

A. Sample treatment

Note: Add 1M Dithiothreitol (DTT) to 1x mRNA Binding Buffer to make a final concentration of 10 mM of DTT.

A1. Animal/Plant tissues

1. Wholly and quickly homogenize the desired amount of plant or animal tissue in liquid nitrogen.
2. Collect and transfer the frozen powder to a fresh tube. Add 1ml 1x mRNA Binding Buffer per 100 mg tissue, mix well by inverting the tube several times and incubate at room temperature for 5 min with rotational mixing.
3. Reduce solution viscosity by shearing DNA through a syringe with an 18-gauge needle by sucking it in and out several times.
4. Centrifuge at 14,000 rpm for 5 min at room temperature and transfer all the supernatant to a
5. fresh tube. The supernatant is ready for mRNA purification or can be stored at –80ºC for future use.

A2. Cell suspension

1. Pellet cells by centrifugation at 4,000 rpm for 5 min at room temperature and discard the supernatant. Add 1 ml 1x mRNA Binding Buffer per 5 x 10^6 - 10 x 10^6 animal or plant cells.
2. Lyse cells by pipetting several times until the solution becomes viscous. Reduce solution viscosity by shearing DNA through a syringe with an 18-gauge needle by sucking it in and out several times.
3. Centrifuge at 140 00 rpm for 5 min at room temperature and carefully transfer supernatant to a fresh tube. The supernatant is ready for mRNA purification or can be stored at –80ºC for future use.

A3. Yeast and Fungi

1. Harvest yeast or fungi cells from the desired amount of culture by centrifugation at 14,000 rpm for 6 min at room temperature and remove all the supernatant. Quickly flash-freeze sample by immersion in liquid nitrogen bath.
2. Add 1 ml 1x mRNA Binding Buffer per 1.5 ml culture and let the sample thaw on ice. Add 70 µl glass beads (sigma Cat# G-8772) per 1.5 ml culture and place them in a vortex mixer for 3 min.
3. Centrifuge at 14,000 rpm for 5 min at room temperature and transfer all the supernatant to a fresh tube.
4. Reduce solution viscosity by shearing DNA through a syringe with an 18-gauge needle by sucking it in and out several times.
5. Centrifuge at 14,000 rpm for 5 min at room temperature and carefully transfer supernatant to a fresh tube. The supernatant is ready for mRNA purification or can be stored at –80ºC for future use.

A4. Total RNA

1. The concentration of total RNA should be adjusted to ~1 µg/µl with ultrapure water. Divide the RNA sample into aliquots of 100 µl per tube, heat at 70°C for 5 min to destroy the RNA secondary structure, and immediately chill on ice.
2. Add 1ml 1x Binding buffer to the RNA solution.

B. mRNA purification

1. Gently shake the bottle of BcMag•mRNA beads until the magnetic beads are completely suspended and transfer the desired amount of the beads to a fresh tube.

Note: Use 100µl of BcMag oligo-d$(T)_{30}$ magnetic beads per 100 mg tissue or 1.5 ml yeast/fungi or 107 cells or 200µg total RNA.

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2. Place the tube in a magnetic Rack and wait for 1-3 min until the supernatant becomes clear.
3. Remove and discard the supernatant. Suspend the beads with 0.5 volumes of 1x Binding Buffer.
4. Combine the magnetic beads with the pre-treated sample or the sample stored at 80ºC as described above.
   Note: Samples previously stored at –80ºC should first be thawed on ice.
5. Remove the tube from the magnetic Rack, mix the beads very well and incubate at room temperature for 10 min with rotational mixing.
6. Place the tube in the magnetic Rack, wait 1 min and discard the supernatant. Resuspend the beads with four volumes of 1x Washing buffer I.
7. Repeat (step 5) once.
8. Place the tube in the magnetic Rack, wait 1 min and discard the supernatant. Resuspend the beads with four volumes of 1x Washing buffer II.
9. Repeat (step 8) once.
10. Place the tube in the magnetic Rack, wait 1min and discard the supernatant completely.
11. Carefully transfer the mRNA-containing supernatant to a fresh tube. Heat the tube at 65°C for 3 min and immediately separate the beads from the released mRNA by the magnetic Rack for 2 min. Add 20µl elution buffer, remove the tube from the magnetic Rack, and mix well.
    Note:
    - If mRNA is used for cDNA synthesis, the magnetic beads should be washed with 100 µl 1x Reverse Transcription buffer one time after step 9. The mRNA can be eluted by adding the desired amount of 1x Reverse Transcription buffer, heated at 65ºC for 3 min and collected by magnetic Rack. or the bonded mRNA
    - beads can be directly used in reverse transcription reactions.
    - Or the mRNA/bead complex can also be directly used for cDNA synthesis without elution after step 9. The
    - mRNA/bead complex should be washed one time with 100 µl 1x Reverse Transcription buffer, and then suspend mRNA/bead complex in the desired amount of 1x Reverse transcription buffer.

C. Storage

The eluted mRNA solution can be stored at –20°C for a short time. For long-term storage, the mRNA solution should be stored in 75% ethanol at -80°C.

Troubleshooting

Problem	Probable cause	Suggestion
The yield of purified mRNA is low or undetectable. •	Sample homogenization is incomplete.	• Try different methods to homogenize samples and increase homogenization time.
	mRNA is not completely dissolved.	• Incubate at 70ºC for 5 min. For highly viscous samples, add more ultrapure water pipettes several times. mRNA degradation will decrease the yield and can be detected by denaturing agarose gel electrophoresis.
	The pre-treated sample is too viscous. If the pre-treated sample is too viscous, it will influence the efficiency of the beads' binding to mRNA.	• Repeat shearing DNA through a syringe with an 18-gauge needle by sucking it in and out several more times until the sample loses viscosity.
	mRNA degradation .	• Completely and quickly homogenize tissues or cells. Use fresh samples, or quickly freeze them with liquid nitrogen and store them at –80ºC. • RNAse contamination. Use new, sterile disposable centrifuge tubes. Always wear disposable gloves and change frequently.

		Use RNase-free water for solution preparation. Glassware should be baked at 300ºC for four h or 180ºC for eight hours.
Problem: Ribosomal RNA (rRNA) contamination	The purified mRNA from some samples may carry over a small amount of rRNA. For some applications, such as Northern blots, the contamination will not significantly affect the experimental results. However, if the mRNA is used.	For cDNA library construction, rRNA should be eliminated from the mRNA solution. Usually, rRNA can be removed by repeating the same purification method used for the total RNA once more.

Related products

Products and Catalog Number	
Genomic DNA and RNA Purification	
One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Step Saliva Viral RNA-DNA Purification Kit, Cat. No. AR101
Cell-Free DNA Purification Kit, Cat. No AC101	Bone-Teeth DNA Purification Kit, Cat. No. AB101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ-101	Rootless Hair DNA Purification Kit, Cat. No. AD101
One-Step Bacteria DNA Purification Kit, Cat. No. AE101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Touch DNA Purification Kit, Cat. No. AS101
One-Step Fungi &Yeast DNA Purification Kit, Cat. No. AL101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101
One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101	One-Step Dandruff DNA Purification Kit, Cat. No. AAA101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	Quick mRNA Purification Kit, Cat. No. MMS101
DNA & RNA Sample Preparation	
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step DNA-RNA Removal Kit, Cat. No. CA103
One-Step RNA Removal Kit, Cat. No. AU101	One-Step DNA/RNA Cleanup Kit, Cat. No. AH101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
Quick Oligo-DNA Conjugation Kit, Cat. No. CA101	One-Step Fluorescent Labeling Cleanup Kit, Cat. No. AK101
One-Step DNA-RNA Removal Kit, Cat. No. AV101	One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101
One-Step PCR Inhibitor Removal Kit, Cat. No. AX101	Pure Miniprep Plasmid DNA Purification Kit, Cat. No. AY101