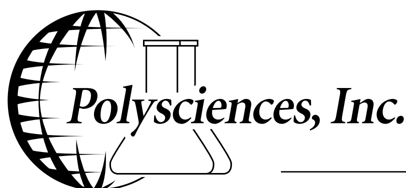

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BioMag® SelectaPure™ mRNA Purification System

Catalog # 8MB4003K

1. Product Description

Polysciences BioMag® SelectaPure™ mRNA Purification System offers a rapid, low-cost method for isolating mRNA using BioMag superparamagnetic particles linked to oligo $(dT)_{20}$ using a proprietary linker technology. The BioMag Oligo $(dT)_{20}$ particles are approximately 1 µm in size and are provided in a ribonuclease-free suspension.

Under the appropriate conditions, the poly dT covalently linked to the surface of the BioMag particle base pairs with the poly A residues at the 3' end of most mRNA. Once bound, the particle - mRNA complex is removed from solution by applying an external magnetic field. The supernatant is discarded and the mRNA is eluted from the particles with DEPC-treated water.

Product Characteristics

Isolates	• 1 to 5 µg of mRNA from approximately 100 µg of total RNA or from 5 x 10^6 cells • 3 to 15 µg from 50 to 100 mg tissue, depending on tissue type and quality
Sample size	• 100 µg total RNA • 50 to 100 mg tissue • 5 x 10^6 cells
Number of isolations	• 20 isolations from total RNA • 10 isolations from tissue or cell lysates
Approximate time required for purification	• 15 minutes from total RNA • 45 minutes from tissue or cell lysates

2. Safety Precautions

When handling any chemicals in this System, observe all relevant precautions, and follow all state, local, and federal regulations for chemical handling and disposal.

3. Materials

Store the lyophilized proteinase K, the 2-mercaptoethanol, and the BioMag Oligo $(dT)_{20}$ particles at 4 °C. Store the rest of the kit at room temperature.

3.1 Materials Provided

BioMag Oligo $(dT)_{20}$ (cat.#8MB4803N) — 1 vial (2 ml) (5 mg/ml) in 20 mM Tris, pH 7

2-Mercaptoethanol — 1 vial (0.5 ml)

Urea Lysis Buffer — 1 vial (25 ml)
Composition: 4M Urea, 0.1M Tris pH 8, 0.5M NaCl, 10mM EDTA, 1% SDS
Notes: The Lysis Buffer may form a precipitate in storage. To redissolve, warm it before use.

Lyophilized Proteinase K — 1 vial (10mg)
Store at 4 °C until solubilization. To use, resuspend in 0.5 ml DEPC water (to 20 mg/ml) and store at -20 °C

Binding Buffer — 1 vial (50ml)
Composition: 20 mM Tris (pH 8.0), 0.5M NaCl

Wash Buffer — 1 vial (25ml)
Composition: 7mM Tris (pH 8.0), 0.17M NaCl

DEPC-treated water — 2 vials (2ml)
Composition: Water treated with 0.1% DEPC

5 M NaCl — 1 vial (1ml)
Notes: The 5 M NaCl may form a precipitate in storage. To redissolve, warm it before use.

Antifoam A — 1 vial (250µl)

3.2 Materials Needed But Not Provided

To purify mRNA with the BioMag mRNA Purification System, you need a magnetic separator. The procedures in these Operating Instructions were developed for use with either of the following Polysciences magnetic separators:

- Solo-Sep Microcentrifuge Tube Separator (single sample)— Part Number 8MB4112S
- Multi-6 Microcentrifuge Tube Separator (six samples)— Part Number 8MB4111S

Other rare-earth magnetic separators may work, but due to their different specifications, you may have to adapt separation time to get satisfactory results.

In addition, you need:

- Nuclease-free microcentrifuge tubes
- Water bath or heating block set to 60 °C

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Tissue and cell samples also require:

- Centrifuge capable of 14,000 x g
- Tissue homogenizer
- 20-gauge needle and syringe
- Water bath or heating block set to 56°C
- 1X PBS

4. Minimizing Ribonuclease Contamination

It is important to minimize sources of ribonuclease contamination in the laboratory. Whenever possible:

- Use sterile, disposable plasticware.
- Bake laboratory glassware at 200°C overnight.
- Make buffers with sterile autoclaved DEPC (diethyl pyrocarbonate) treated water.
- Wear disposable gloves at all times and change them frequently during the experiment, as your hands can be a major source of ribonucleases.

5. Washing BioMag Oligo $(dT)_{20}$ Particles

1. Shake the BioMag Oligo $(dT)_{20}$ vial to ensure uniform suspension.
2. Dispense 100 µl (for total RNA) or 200 µl (for tissues or cells) of BioMag Oligo $(dT)_{20}$ into a microcentrifuge tube.
3. Place on a magnetic separator until the supernatant is clear.
4. While the tube is on the separator, aspirate and discard the supernatant. Be careful not to remove any particles with the supernatant. See the Helpful Tip for Aspirating Supernatant below.
5. Wash the particles:
   - Resuspend in 200 µl of Binding Buffer by pipet.
   - Place on a magnetic separator until the supernatant is clear.
   - Aspirate and discard the supernatant.
6. Resuspend the particles in 100 µl (for total RNA) or 200 µl (for tissues or cells) of Binding Buffer.

Helpful Tip for Aspirating Supernatant

It is important not to remove any particles with the supernatant. To aspirate the most supernatant without particles:

1. Hold the separator with the tube at eye level.
2. Tilt the tube such that the magnet wall of the separator faces up.
3. Hold the tube firmly against the wall. Angle the pipet tip away from the wall of the tube against which the magnetic particles rest and aspirate the supernatant.

5.1 Purifying mRNA From Total RNA

Use this procedure for isolation from a total RNA preparation. Section 6 provides a procedure for use with tissue or whole cells. Purifying mRNA from tissue and cell samples involves three steps:

1. Binding the mRNA.
2. Eluting the mRNA.

5.2 Binding the mRNA

1. Bring the volume of the total RNA sample up to 90 µl with DEPC-treated water.
2. Incubate the RNA sample at 60 °C for 5 minutes.
3. Add 10 µl of 5M NaCl for a final concentration of 0.5M NaCl. If the NaCl precipitated in storage, redissolve it by warming before use.
4. Add the RNA to the tube containing the washed BioMag particles (from Section 5, Washing BioMag Oligo $(dT)_{20}$ Particles.)
5. Mix gently and let hybridize at room temperature for 3 to 5 minutes.
6. Place on a magnetic separator until the supernatant is clear. This should take just a few minutes. Discard the supernatant.
7. Wash the mRNA bound particles:
   - Resuspend in 100 µl of Wash Buffer and pipet up and down to mix.
   - Place on a magnetic separator until the supernatant is clear.
   - While the tube is on the separator, aspirate and discard the supernatant.
8. Repeat Step 7.

5.3 Eluting the mRNA

1. Resuspend the bound mRNA particles in 40 to 50 µl of DEPC-treated water.
2. Incubate at 60 °C for 2 minutes.
3. Place on a magnetic separator until the mRNA supernatant is clear.
4. Transfer the supernatant containing the eluted mRNA to a nuclease-free microcentrifuge tube. Store at -70 °C.

For long-term storage, you can prevent contamination and loss of material due to RNases by adding either:

- 1U/µl placental RNase inhibitor with a final dithiothreitol concentration of 1mM.
- SDS to a final concentration of 0.5%. SDS may interfere with some applications.

6. Purifying mRNA From Tissue and Cell Samples

Use this procedure to isolate mRNA from tissue and cell samples. Section 5 contains a procedure for use with total RNA.

Purifying mRNA from tissue and cell samples involves three steps:

1. Preparing tissue or cell lysates.
2. Binding the mRNA.
3. Eluting the mRNA.

6.1 Preparing Tissue Lysates

To prepare a tissue lysate sample:

1. Prepare the Working Lysis Buffer by adding 20 µl Proteinase K stock solution, 10 µl 2-Mercaptoethanol, and 6 µl Antifoam A to 1 ml stock Urea Lysis Buffer. If the buffer precipitated in storage, warm to redissolve it before use.
2. Pulverize the tissue sample in liquid nitrogen using a ceramic mortar and pestle. If you are using freshly dissected tissue, freeze it immediately in liquid nitrogen before weighing it.
3. Transfer the frozen powder to the homogenizer vessel using 1 ml Working Lysis Buffer per 100 mg tissue.
4. Homogenize 1 to 3 minutes to ensure complete lysis of cells.

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5. Incubate the lysate at 56 °C for 15 minutes to allow protein digestion.

Note: At this point, the lysate (or a portion of it) can be aliquoted and stored at -70 °C until needed. Work with lysate on ice.

6. Centrifuge lysate at 14,000 x g for 10 minutes. While centrifuging, proceed to Section 5, Washing BioMag Oligo $(dT)_{20}$ Particles.

6.2 Preparing Cell Lysates

Use this procedure to prepare cell lysates from a cell monolayer or from a cell suspension.

Cell monolayer

1. Prepare the Working Lysis Buffer by adding 20 µl Proteinase K stock solution, 10 µl 2-Mercaptoethanol, and 6 µl Antifoam A to 1 ml stock Urea Lysis Buffer. If the buffer precipitated in storage, warm to redissolve it before use.
2. Wash the cell monolayer with 1X PBS.
3. Scrape the culture directly in the Working Lysis Buffer (5 to 10 x10^6 cells/ml).
4. Draw the lysate solution up and down through a 20-G needle into a syringe a few times to ensure complete lysis.
5. Transfer lysate to a tube and incubate at 56 °C for 15 minutes.
6. Centrifuge the sample homogenate at 14000 x g for 10 minutes. While centrifuging, proceed to Section 5, Washing BioMag Oligo $(dT)_{20}$ Particles.

Cell suspension

1. Prepare the Working Lysis Buffer by adding 20 µl Proteinase K stock solution, 10 µl 2-Mercaptoethanol, and 6 µl Antifoam A to 1 ml stock Urea Lysis Buffer. If the buffer precipitated in storage, warm to redissolve it before use.
2. Centrifuge the cells at 300 x g for 5 minutes.
3. Wash the cell pellet by resuspending in 1X PBS equal to half the volume of the original cell suspension and centrifuging.
4. Resuspend the pellet in Working Lysis Buffer (5 to 10 x10^6 cells/ml). Draw the lysate solution up and down through a 20-gauge needle into a syringe a few times to ensure complete lysis.
5. Transfer lysate to a tube and incubate at 56°C for 15 minutes.
6. Centrifuge the sample homogenate at 14,000 x g for 10 minutes. While centrifuging, prepare BioMag Oligo $(dT)_{20}$ particles for binding. Refer to section 5, Washing BioMag Oligo $(dT)_{20}$ Particles.

6.3 Binding the mRNA

To bind the mRNA:

1. Carefully transfer the supernatant to the tube containing the washed BioMag Oligo $(dT)_{20}$ particles. Be careful not to transfer any cellular debris.
2. Resuspend the BioMag particles in the lysate supernatant by pipeting the solution up and down.
3. Let the supernatant hybridize to the washed BioMag Oligo $(dT)_{20}$ at room temperature for 3 to 5 minutes.
4. Place on a magnetic separator until the supernatant is clear.
5. While the tube is on the separator, aspirate and discard the supernatant.
6. Wash the mRNA bound particles:
   - Resuspend the BioMag Oligo $(dT)_{20}$ particles in 200 µl of Wash Buffer.
   - Place on a magnetic separator until the supernatant is clear.
   - While the tube is on the separator, aspirate and discard the supernatant.
7. Repeat step 6

6.4 Eluting the mRNA

To elute the bound mRNA:

1. Resuspend the mRNA bound particles in 40 to 50 µl of DEPC-treated water.
2. Incubate at 60 °C for 2 minutes.
3. Place on a magnetic separator until the supernatant is clear.
4. Transfer the mRNA supernatant to a clean tube. Store at -70°C.

For long-term storage, you can prevent contamination and loss of material due to RNases by adding either:

- 1U/µl placental RNase inhibitor with a final dithiothreitol concentration of 1mM.
- SDS to a final concentration of 0.5%. SDS may interfere with some applications.

7. Scaling the Procedure

The procedure can be scaled up or down. For tissue quantities greater than 100 mg, increase the quantity of BioMag Oligo $(dT)_{20}$ proportionately (for example, 500 µl for up to 250 mg of tissue).

Note: If a sample contains more than 250 mg of tissue, DNA may be bound non-specifically. To remove this genomic DNA, bind the mRNA to fresh BioMag Oligo $(dT)_{20}$ particles and elute with DEPC-treated water.

8. References

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Technical Support:

Polysciences, Inc., offers a full line of BioMag Purification Systems and particles and is dedicated to helping you use BioMag mRNA Purification Systems to their fullest extent.

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All Technical Data Sheets for BioMag products are available at www.polysciences.com

Ordering Information:

RNA isolation

Catalog #	Description	Size/Format
8MB4003K	**BioMag SelectaPure mRNA Purification System**	20 isolations from total RNA 10 isolations from tissue or cell lysates
8MB4803N	**BioMag Oligo $(dT)_{20}$ Particles** Technical Data Sheet #529	2 ml

DNA/RNA isolations

Catalog #	Description	Size/Format
8MB4804B	**BioMag Streptavidin** Technical Data Sheet #530	10 ml at 1 mg/ml of BioMag
8MB4804C		25 ml at 1 mg/ml of BioMag
8MB4804E		100 ml at 1 mg/ml of BioMag

Magnetic Separators

Catalog #	Description	Size/Format
84101S	**Flask Separator** Technical Data Sheet #571	For use with tissue culture flasks
84102S	**15ml/50ml Tube Separator** Technical Data Sheet #572	Holds five 15ml tubes and three 50ml tubes
84104S	**12 x 75mm Test Tube Separator:** Technical Data Sheet #573	Holds sixty 12 x 75mm tubes
8MB4109S	**96-Well Plate Separator:** Technical Data Sheet #575	For most 96-well plate applications
8MB4112S	**Solo-Sep Microcentrifuge Tube Separator:** Technical Data Sheet #577	Holds one 1.5ml tube
8MB4111S	**Multi-6 Microcentrifuge Tube Separator** Technical Data Sheet #576	Holds six 1.5ml tubes
84106S	**Multi-32 Microcentrifuge Tube Separator:** Technical Data Sheet #574	Holds 32 1.5ml tubes Allows 16 separations at once

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