Product Data Sheet 569

BioMag® mRNA Purification System

9025 Technology Dr. • Fishers, IN 46038-2886
800.387.0672 • 317.570.7020 • Fax 317.570.7034
info@bangslabs.com • www.bangslabs.com

Bangs Laboratories, Inc.

B E A D S ● A B O V E T H E R E S T ™

DESCRIPTION

BioMag® mRNA Purification System offers a rapid, low-cost method for isolating mRNA using BioMag® superparamagnetic particles coated with Oligo dT (20), using a proprietary linker technology. The BioMag® Oligo dT (20) particles are approximately 1.5µm 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.

The BioMag® mRNA Purification System kit provides for 20 isolations from total RNA or 10 isolations from tissue or cell lysates.

CHARACTERISTICS

Mean Diameter:	~1.5µm
Isolates:	1-5µg of mRNA from ~100µg of total RNA or from 5×10^6 cells
	3-15µg from 50-100mg tissue, depending on tissue type and quality
Sample Size:	100µg total RNA
	50-100mg tissue
	5×10^6 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

MATERIAL

Material Supplied

- BioMag Oligo dT (20): 2mL
- 2-Mercaptoethanol: 0.5mL
- Urea Lysis Buffer: 4M Urea, 0.1 Tris (pH 8.0), 0.5M NaCl, 10mM EDTA, 1% SDS: 25mL
  Note: The Lysis Buffer may form a precipitate in storage. To redissolve, warm it before use.
- Lyophilized Proteinase K: 10 mg
  Note: Store at 4˚C until solubilization. To use, resuspend in 0.5mL DEPC-treated water (to 20mg/mL) and store at -20˚C.
- Binding Buffer: 20mM Tris (pH 8.0), 0.5M NaCl: 50mL
- Wash Buffer: 7mM Tris (pH 8.0), 0.17M NaCl: 25mL
- DEPC-treated water: Water treated with 0.1% DEPC: 2mL
- 5M NaCl: 1mL
  Note: The 5M NaCl may form a precipitate in storage. To redissolve, warm it before use.
- Antifoam A: 250µL

Material Required

- Magnetic separator
- Nuclease-free microcentrifuge tubes
- Water bath or heating block set to 60˚C

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

PROCEDURE

Researchers are advised to optimize the use of BioMag® in any application as procedures designed by other manufacturers may not be ideal.

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 ribonuclease.

Washing BioMag® Oligo dT (20) Particles

1. Shake the BioMag® Oligo dT (20) vial to ensure uniform suspension.
2. Dispense 100µL (for total RNA) or 200µL (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 *Helpful Tips for Aspirating Supernatant* below.
5. Wash the particles.
   a. Resuspend in 200µL of Binding Buffer by pipet.
   b. Place on a magnetic separator until the supernatant clears.
   c. Aspirate and discard the supernatant.
6. Resuspend the particles in 100µL (for total RNA) or 200µL (for tissues and cells) of Binding Buffer.

Helpful Tips for Aspirating Supernatant

It is important not to remove any particles with the supernatant. To aspirate the most supernatant without particles, follow these steps.

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.

Purifying mRNA from Total RNA

Use this procedure for isolation from total RNA. The next section provides a procedure for use with tissue or whole cells.

1. Binding the mRNA.
   a. Bring the volume of the total RNA sample up to 90µL with DEPC-treated water.
   b. Incubate the RNA sample at 60˚C for 5 minutes.
   c. Add 10µL of 5M NaCl for a final concentration of 0.5M NaCl. *Note:* If the NaCl precipitated in storage, redissolve it by warming before use.
   d. Add the RNA to the tube containing the washed BioMag® particles (from Step 5, Washing BioMag® Oligo dT (20) Particles).
   e. Mix gently and let hybridize at room temperature for 3-5 minutes.
   f. Place on a magnetic separator until the supernatant is clear. This should take just a few minutes. Discard the supernatant.
   g. Wash the mRNA bound particles.
      i. Resuspend in 100µL of Wash Buffer and pipet up and down to mix.
      ii. Place on a magnetic separator until the supernatant is clear.
      iii. While the tube is on the separator, aspirate and discard the supernatant.
   h. Repeat Step g.

2. Eluting the mRNA.
   a. Resuspend the bound mRNA particles in 40-50µL of DEPC-treated water.
   b. Incubate at 60˚C for 2 minutes.
   c. Place on a magnetic separator until the mRNA supernatant is clear.
   d. Transfer the supernatant containing the eluted mRNA to a nuclease-free microcentrifuge tube. Store at -70˚C. *Note:* 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.

Purifying mRNA from Tissue and Cell Samples

Use this procedure to isolate mRNA from tissue and cell samples. Purifying mRNA from tissue and cell samples involves three steps: preparing tissue or cell lysate samples, binding the mRNA, and eluting the mRNA. The previous section contains a procedure to use with total RNA.

1. Preparing *Tissue or Cell Lysate* Samples.
   a. Preparing Tissue Lysate Sample.
      i. Prepare the Working Lysis Buffer by adding 20µL Proteinase K stock solution, 10µL 2-Mercaptoethanol, and 6µL Antifoam A to 1mL stock Urea Lysis Buffer. *Note:* If the buffer precipitated in storage, warm to redissolve it before use.
      ii. 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.
      iii. Transfer the frozen powder to the homogenizer vessel using 1mL Working Lysis Buffer per 100mg tissue.
      iv. Homogenize 1-3 minutes to ensure complete lysis of cells.
      v. 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.
      vi. Centrifuge lysate at 14,000 x g for 10 minutes. While centrifuging, proceed to Step 5 of the *Washing BioMag® Oligo dT (20) Particles* section above.
   b. Preparing *Cell Lysate* Sample.
      Use this procedure to prepare cell lysates from a cell monolayer or from a cell suspension.
      From a cell monolayer:
      i. Prepare the Working Lysis Buffer by adding 20µL Proteinase K stock solution, 10µL 2-Mercaptoethanol, and 6µL Antifoam A to 1mL stock Urea Lysis Buffer. *Note:* If the buffer precipitated in storage, warm to redissolve it before use.
      ii. Wash the cell monolayer with 1X PBS.
      iii. Scrape the culture directly in the Working Lysis Buffer (5 to 10 x 10^6 cells/mL).
      iv. Draw the lysate solution up and down through a 20-gauge needle into a syringe a few times to ensure complete lysis.
      v. Transfer lysate to a tube and incubate at 56˚C for 15 minutes.
      vi. Centrifuge the sample homogenate at 14,000 x g for 10 minutes. While centrifuging, proceed to Step 5 of the *Washing BioMag® Oligo dT (20) Particles* section above.
      From a cell suspension:
      i. Prepare the Working Lysis Buffer by adding 20µL Proteinase K stock solution, 10µL 2-Mercaptoethanol, and 6µL Antifoam A to 1mL stock Urea Lysis Buffer. *Note:* If the buffer precipitated in storage, warm to redissolve it before use.
      ii. Centrifuge the cells at 300 x g for 5 minutes.
      iii. Wash the cell pellet by resuspending in 1X PBS equal to half the volume of the original cell suspension and centrifuging.
      iv. Resuspend the pellet in Working Lysis Buffer (5 to 10 x 10^6 cells/mL).
      v. Draw the lysate solution up and down through a 20-gauge needle into a syringe a few times to ensure complete lysis.
      vi. Transfer lysate to a tube and incubate at 56˚C for 15 minutes.
      vii. Centrifuge the sample homogenate at 14,000 x g for 10 minutes. While centrifuging, proceed to Step 5 of the *Washing BioMag® Oligo dT (20) Particles* section above.

2. Binding the mRNA.
   a. Carefully transfer the supernatant to the tube containing the washed BioMag® Oligo dT (20) particles. Be careful not to transfer any cellular debris.
   b. Resuspend the BioMag® particles in the lysate supernatant by pipeting the solution up and down.
   c. Let the supernatant hybridize to the washed BioMag® Oligo dT (20) at room temperature for 3-5 minutes.
   d. Place on a magnetic separator until the supernatant is clear.
   e. While the tube is on the separator, aspirate and discard the supernatant.
   f. Wash the mRNA bound particles.
      i. Resuspend the BioMag® Oligo dT (20) particles in 200µL of

Wash Buffer.
ii. Place on a magnetic separator until the supernatant is clear.
iii. While the tube is on the separator, aspirate and discard the supernatant.
g. Repeat Step f.

3. Eluting the bound mRNA.
   a. Resuspend the bound mRNA particles in 40-50µL of DEPC-treated water.
   b. Incubate at 60˚C for 2 minutes.
   c. Place on a magnetic separator until the mRNA supernatant is clear.
   d. Transfer the mRNA supernatant to a clean tube. Store at -70˚C. *Note:* 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.

Scaling the Procedure

This procedure can be scaled up or down. For tissue quantities greater than 100mg, increase the quantity of BioMag® Oligo dT (20) proportionately (for example, 500µL for up to 250mg of tissue). *Note:* If sample contains more than 250mg of tissue, DNA may be bound nonspecifically. To remove this genomic DNA, bind the mRNA to fresh BioMag® Oligo dT (20) particles and elute with DEPC-treated water.

REFERENCES

1. **Hengerer, B.** 1993. A rapid procedure for mRNA extraction from a large number of samples. *BioTechniques*, 14(4):522-524.
2. **Morrissey, D.V., M. Lombardo, J.K. Eldredge, K.R. Kearney, E.P. Goody, M.L. Collins.** 1989. Nucleic acid hybridization assays employing dA-tailed capture probes. Multiple capture methods. *Anal Biochem*, 181(2):345-359.
3. **McKendree Jr., W.L., C.J. Nairn III, M.G. Bausher.** 1995. Differential displays from plant leaves using oligo (dT) magnetic bead mRNA isolation and hot air PCR. *BioTechniques*, 19(5):715-716, 719.

STORAGE AND STABILITY

Store the lyophilized Proteinase K, the 2-Mercaptoethanol, and the BioMag® Oligo dT (20) at 2-8˚C. The rest of the kit may be stored at room temperature. Freezing, drying, or centrifuging BioMag® may result in irreversible aggregation and loss of binding activity.

These products are for research use only and are not intended for use in humans or for *in vitro* diagnostic use.

ORDERING INFORMATION

Cat. Code	Description	Sizes
BM569	BioMag® mRNA Purification System	1 kit
BM529	BioMag® Oligo dT (20), Nuclease-free	2mL

Order online anytime at **www.bangslabs.com.**