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Products for Biotechnology

With Magnetic Porous Glass (MPG®)

Product: **MPG® Guanidine Direct mRNA Purification Kit**
Product No: **MGRK1010**
Procedure: **Isolation and purification of mRNA directly from cells, animal or plant tissue.**
Kit Storage: **Stable for 1 year at 4º C, DO NOT FREEZE**

The **MPG® Guanidine Direct mRNA Purification Kit** is designed specifically for the isolation of mRNA directly from cells, animal and plant tissue containing high levels of RNases. The Guanidine Thiocyanate Homogenization Buffer (GTC) included in this kit is integral for inactivating the stubborn RNases thus preventing RNA degradation. Direct mRNA isolation eliminates the need for an initial total RNA purification step, use of organic solvents or Oligo dT cellulose columns. The isolation takes 15 minutes or less.

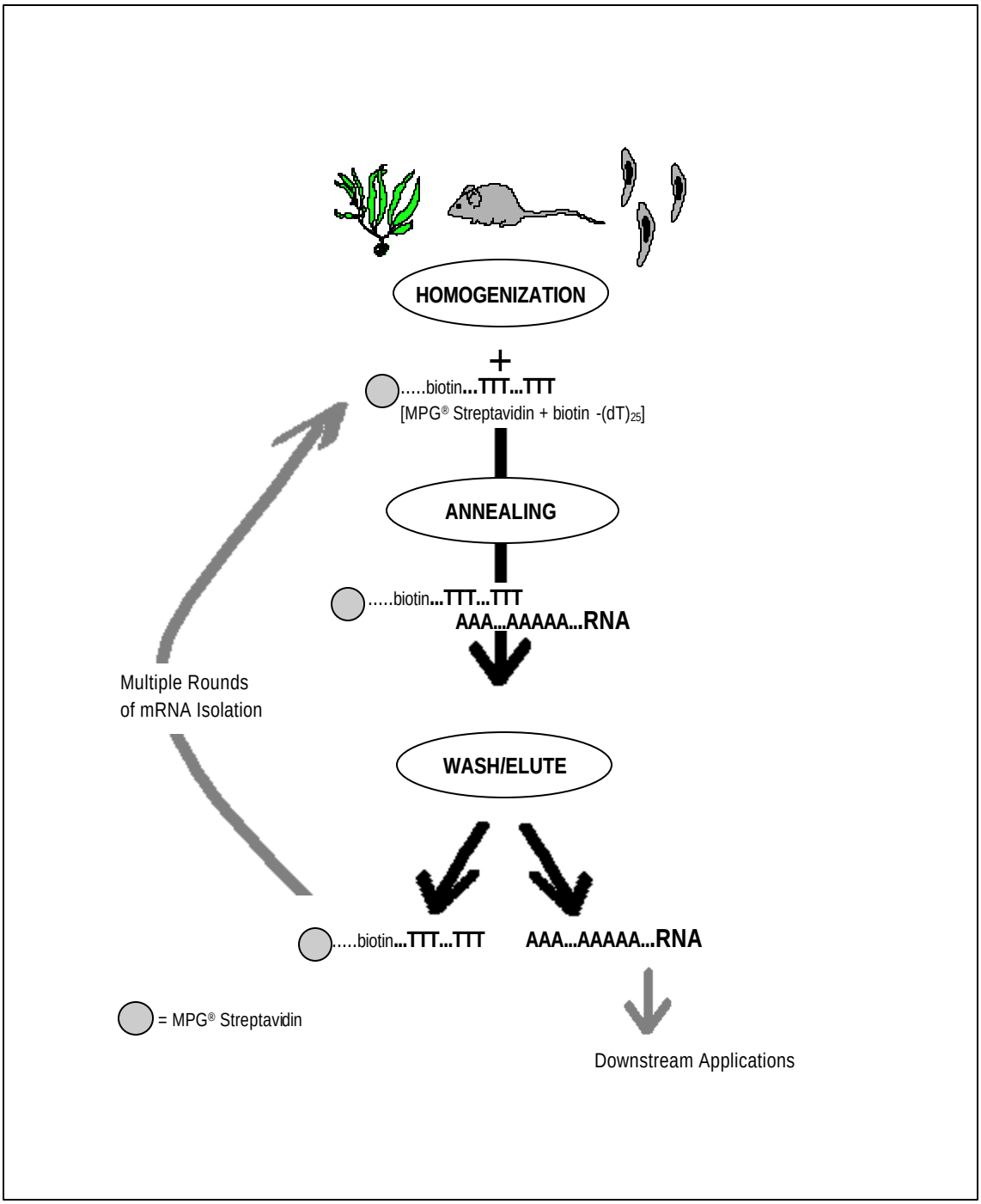
The purified mRNA is compatible with downstream applications in molecular biology including:

- cDNA Library Construction
- Subtractive Hybridization
- S1 Nuclease Analysis
- Dot Blot Hybridization
- Cloning
- Northern Blotting
- *in vitro* Translation
- Sequencing
- RNA Binding Protein Isolation
- RT-PCR

MPG® Guanidine Direct mRNA Purification Kit contains 1 ml (10 mg) of MPG® Streptavidin Biotinylated Oligo $(dT)_{25}$ complex (MPG® Streptavidin Complex). MPG® Streptavidin Complex is an aqueous suspension of 5 micrometers diameter, 50 nanometers (500 Angström) pore diameter, superparamagnetic, totally porous glass particles with streptavidin covalently coupled to its surface and preloaded with Biotinylated Oligo $(dT)_{25}$ at a level optimized for maximal mRNA yield. In addition, the MPG® Guanidine Direct mRNA Purification Kit contains all the reagents necessary to isolate and purify at least 30 µg of high quality mRNA (using mouse liver) from a minimum of 1.0 gram of tissue. The upper limit on capacity is approximately 7 µg mRNA/ mg MPG® Streptavidin Complex.

Kit Contains:

1 ml	MPG® Streptavidin Biotinylated Oligo $(dT)_{25}$ Complex (10 mg, suspended in 50 mM Tris HCl, pH 7.2, 2.0 M NaCl, 0.02% NaN_3)
20 ml	GTC Homogenization Buffer
2 X 20 ml	Hybridization Binding Buffer
30 ml	Hybridization Wash Buffer
1 ml	Release Solution
200 µl	ß-Mercaptoethanol



Schematic Diagram Illustrating the Procedure for isolation and purification of mRNA directly from plant tissue, animal tissue and cells accomplished in a single tube by magnetic separation technology. The purified mRNA (A_{260}/A_{280} > 1.8) is suitable for downstream applications.

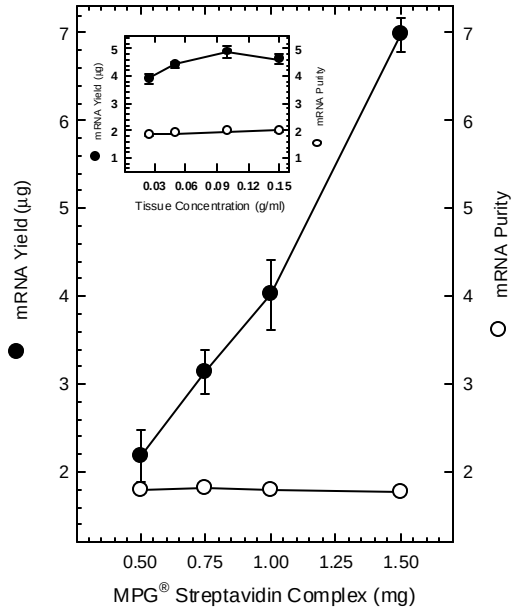
General Procedure

Materials: (Needed but not supplied)

Tissue of Interest
Magnetic Particle Separator, Product No. MPS0301 or MPS0001
Mini-Homogenizer or Polytron
Microcentrifuge
65°C Water Bath
UV/Vis Spectrophotometer
1.5 ml Nuclease-free Microcentrifuge Tubes
50 ml Sterile Screw Cap Conical Tubes
Vortex Mixer
Nuclease-free Pipettes and Pipette Tips
Low Speed Rotator

TECHNICAL TIPS:

TIP #1: To obtain maximum mRNA yield and purity keep the concentration of tissue or cells to Hybridization Binding Buffer between 0.05 - 0.1 g/ml.



Titration of MPG® Streptavidin Complex. mRNA was isolated from 1 ml aliquots of mouse liver homogenates (0.05 g/ml Tissue Extraction/Hybridization Buffer) with increasing amounts of MPG® Streptavidin Complex. 4 µg mRNA was isolated per mg of MPG® Streptavidin Complex. Inset: **Titration of tissue**. mRNA was isolated from samples containing increasing concentrations of mouse liver to Tissue Extraction/Hybridization Buffer with 1 mg MPG® Streptavidin Complex. Tissue concentrations greater than 0.1 g/ml decreased mRNA yield (mRNA was isolated using protocol 5.2).

TIP #2: The yield of mRNA isolated is dependent on the origin of the tissue.

SAMPLE mouse tissue	*Yield mRNA µg/mg MPG® Streptavidin Complex*	*Purity mRNA A_{260}/A_{280}*
Liver	4.0	1.9
Brain	3.4	1.8
Kidney	3.4	1.8
Lung	3.4	1.8

mRNA yield is dependent on the origin of the tissue. Freshly isolated mouse tissues were homogenized in Tissue Extraction/Hybridization Buffer (0.05 g/ml). 1 ml homogenate was added to 1 mg MPG® Streptavidin Complex and mRNA was isolated (using protocol 5.2).

TIP #3: *Successful isolation of intact mRNA requires that endogenous Ribonuclease (RNase) activity be minimized and that reagents and labware be free of RNase contamination. RNases are ubiquitous and highly resistant to chemical and temperature degradation. It is advisable to become thoroughly familiar with the techniques for handling RNA, and for minimizing and remediating RNase contamination, by consulting a general reference. Suggested sources include J. Sambrook, E.F. Fritsch and T. Maniatis (1989)* ***Molecular Cloning****:* ***A Laboratory Manual****, 2nd Edition. pp. 7.3-7.5 and S.L. Berger and A.R. Kimmel, eds. (1987)* ***Methods in Enzymology: Guide to Molecular Cloning Techniques****,* ***152****, pp. 215-304 and the references contained therein.*

***A REMINDER BEFORE YOU START YOUR PROCEDURE**, THIS PROTOCOL IS BASED ON USING 1 mg MPG® STREPTAVIDIN COMPLEX. 1 mg of MPG® STREPTAVIDIN COMPLEX CAN BIND AN AVERAGE OF 5 µg OF mRNA. THIS PROTOCOL CAN BE SCALED UP OR DOWN BY PROPORTIONALLY ADJUSTING THE COMPONENT VOLUMES PER 1 mg OF MPG® STREPTAVIDIN COMPLEX. OPTIMAL RESULTS WILL BE OBTAINED USING FRESH CELLS OR TISSUE.

****WORK WITH KIT COMPONENTS AT 4°C UNLESS OTHERWISE SPECIFIED IN PROCEDURE BELOW.**

Preparation of MPG® Streptavidin Complex

1. Vortex the MPG® Streptavidin Complex to fully suspend the particles. Transfer 100 µl (1 mg) of MPG® Streptavidin Complex to a 1.5 ml nuclease-free microcentrifuge tube. Magnetically separate using a magnetic particle separator and carefully remove the supernatant.

2. Resuspend the MPG® Streptavidin Complex in Hybridization Binding Buffer (100 µl per mg MPG® Streptavidin Complex) and put it aside until ready for hybridization.

Preparation of Tissue/Cells

1. Prepare GTC Solution by transferring 1.5 ml of GTC Homogenization Buffer into a 50 ml centrifuge tube. Add 15 µl of ß-Mercaptoethanol. Chill this tube on ice.

2. Isolate and weigh 0.1 - 0.25 g fresh tissue of interest (if using cultured cells, harvest 10^6 - 10^7 cells with 0.5 ml GTC Solution). To minimize mRNA degradation, quickly place tissue into the pre-cooled tube from step 1 above. Homogenize for 1-2 minutes. (For cultured cells, lyse the cells directly in GTC Solution). Keep on ice. For isolation of mRNA from plant tissue use 0.1-0.2 g/ml and grind the plant tissue using a mortar and pestle on a -70°C ice bath (liquid nitrogen or acetone - dry ice).

3. Dilute the homogenate with 2 volumes of Hybridization Binding Buffer, (i.e. 3 ml for each 1.5 ml homogenate). Mix well. Centrifuge for 45 seconds to 1.5 minutes at 14,000 x g.

Direct Isolation of mRNA

1. Carefully remove the supernatant from the MPG® Streptavidin Complex (from *Preparation of MPG® Streptavidin Complex* Section, Step 3) and add 1.0 - 1.5 ml tissue supernatant per milligram of the MPG® Streptavidin Complex. Vortex well and incubate with gentle mixing for 3 to 5 minutes. Magnetically separate and carefully remove the supernatant.

2. Resuspend the mRNA-bound MPG® Streptavidin Complex in Hybridization Wash Buffer (1 ml per mg MPG® Streptavidin Complex). Magnetically separate and carefully remove the supernatant. Repeat two more times.

3. Resuspend the mRNA-bound MPG® Streptavidin Complex in Release Solution (20 µl per mg MPG® Streptavidin Complex) and heat at 65°C for 2 minutes. Magnetically separate and carefully transfer the supernatant (which now contains isolated mRNA) to a new 1.5 ml nuclease-free microcentrifuge tube. Repeat this step one more time, pooling the mRNA supernatants, if an additional 10% recovery is desired.

Determination of Yield and Purity of mRNA

Measure the optical density (OD) of the isolated mRNA at wavelengths of 260 nm and 280 nm. (NOTE: It is recommended to use TE Buffer to read OD. Do not use DEPC treated water to read OD, it will lower the A_{260}/A_{280} ratio by 0.2 - 0.3)

Yield of mRNA (µg/ml)=(OD_{260})(40) (dilution factor)
Purity of mRNA = $(OD_{260})/(OD_{280})$

Note: $(OD_{260})/(OD_{280})$ of pure mRNA is $\cong$ 2.0

Recommended Long-Term Storage of Purified mRNA

Store at -70°C. Avoid freeze-thaw cycles.

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