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Products for Biotechnology

With Magnetic Porous Glass (MPG®)

Product: MPG® Direct mRNA Purification Kit
Product No: MDRK1010
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® Direct mRNA Purification Kit is designed specifically for the isolation of mRNA directly from cells and from animal or plant tissue, eliminating the need for an initial total RNA purification step. The isolation takes 15 minutes or less and eliminates the need to use organic solvents or oligo dT cellulose columns.

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

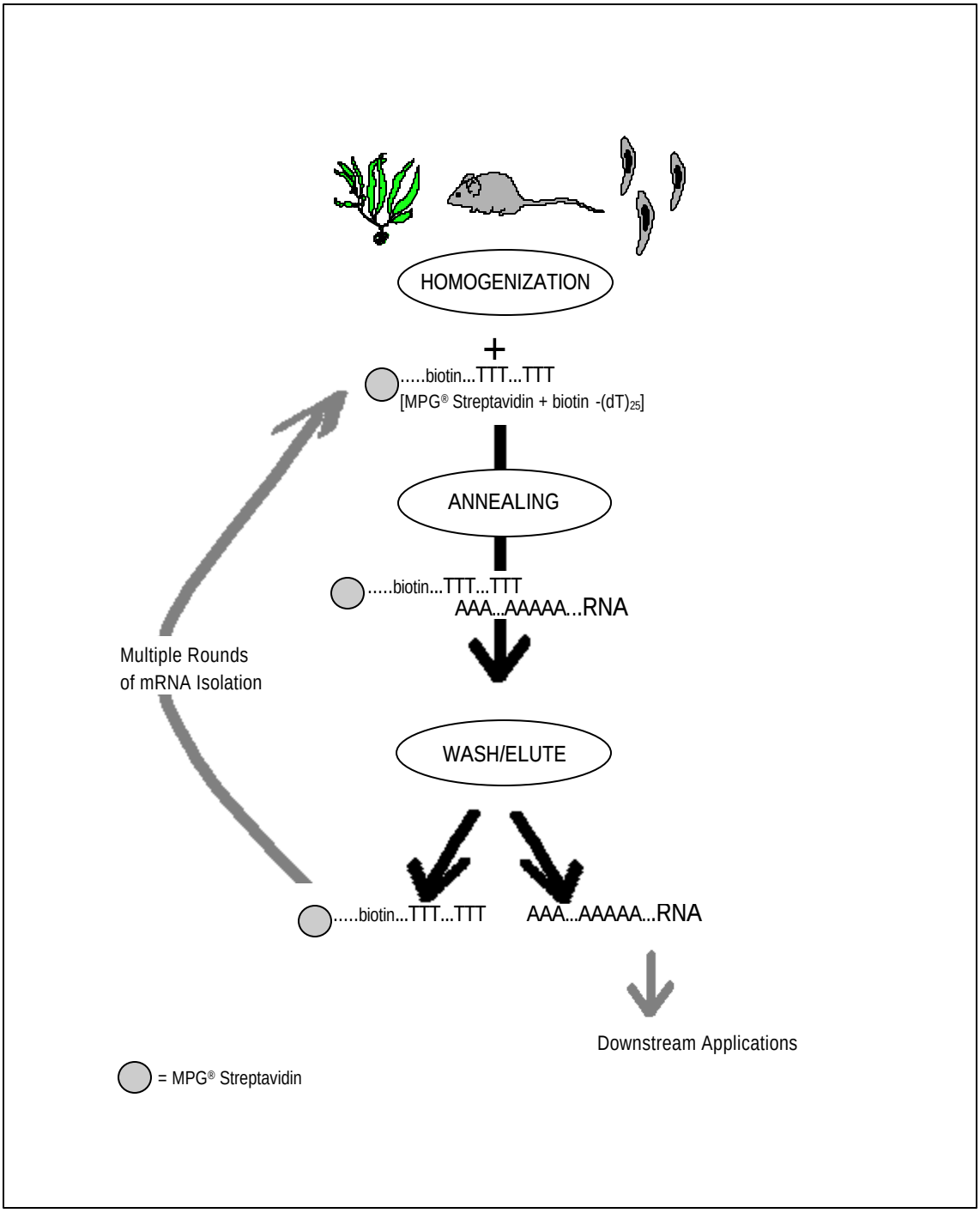
The purified mRNA is compatible with downstream applications in molecular biology including:
MPG® Direct mRNA Purification Kit contains 1 ml (10 mg) of MPG® Streptavidin Biotinylated Oligo $(dT)_{25}$ Complex (MPG® Streptavidin Complex). The MPG® Streptavidin Complex is an aqueous suspension of 5 micrometers diameter, 50 nanometers (500 Ångström) 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® 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 7µg mRNA/mg MPG® Streptavidin Complex.

> Isolation of mRNA from cells or animal tissue containing high levels of RNase may require Guanidine Thiocyanate Homogenization Buffer. MPG® Guanidine Direct mRNA Purification Kit is available.

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 mg	Dithiothreitol
25.0 ml	Tissue Extraction/Hybridization Buffer
20 ml	Hybridization Wash Buffer with LiDS
20 ml	Hybridization Wash Buffer
1 ml	Release Solution

Important: Prior to starting the procedure, add the 20 mg Dithiothreitol to the vial of Tissue Extraction/ Hybridization Buffer provided and mix. Chill on ice for isolation of Tissue/Cells. Keep this Tissue Extraction Hybridization Mixture stored at -20ºC for future isolations. Any precipitant formed due to freezing, will dissolve upon warming.



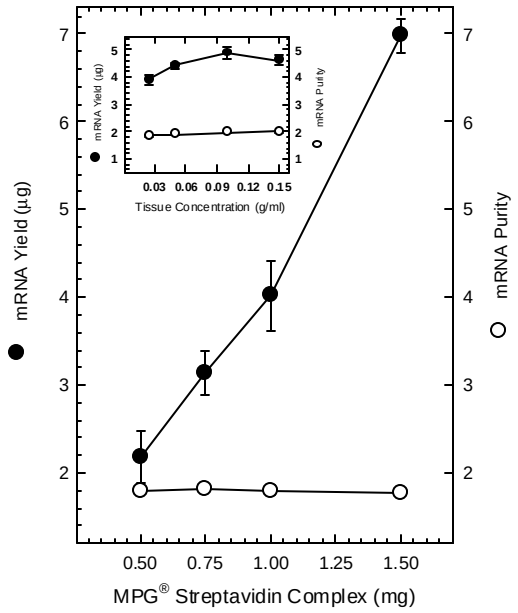
Schematic Diagram Illustrating the Procedure for isolation and purification of mRNA directly from plant tissue, animal tissue and cells accomplished in a single tube by magnetic separation technology. The purified mRNA (A_{260}/A_{280} > 1.8) is suitable for downstream applications.

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 Tissue Extraction/Hybridization 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.

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.

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.

Preparation of MPG® Streptavidin Complex

1. Warm the Kit components to room temperature except the Tissue Extraction/Hybridization Mixture .

2. 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.

3. Resuspend the MPG® Streptavidin Complex in Tissue Extraction/Hybridization Mixture (100 µl per mg MPG® Streptavidin Complex) and put it aside until ready for hybridization.

Isolation of Tissue/Cells

1. Isolate and weigh fresh tissue of interest. To minimize mRNA degradation quickly place the tissue in a chilled 50 ml tube and add enough cold Tissue Extraction/Hybridization Mixture so that the final concentration of tissue is between 0.05-0.10 grams per ml. (If using cultured cells, harvest 10^6-10^7 cells per ml). Homogenize for 1-2 minutes 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).

2. Centrifuge 45 seconds to 1.5 minutes at 14,000 x g.

Direct Isolation of mRNA

1. Magnetically separate and 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 prepared Oligo $(dT)_{25}$-bound MPG® Streptavidin Complex particles. Vortex well and incubate with mixing for 3 to 5 minutes at room temperature. Magnetically separate and carefully remove the supernatant.

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

3. Resuspend the mRNA-bound MPG® Streptavidin Complex in 1 ml Hybridization Wash Buffer. Magnetically separate and carefully remove the supernatant. If the mRNA is to be subsequently reacted with enzymes repeat once more.

4. 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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