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Products for Biotechnology

With Magnetic Porous Glass (MPG®)

Product: MPG® mRNA Purification Kit
Product No: MRRK1010
Procedure: Isolation and purification of mRNA from Total RNA.
Kit Storage: Stable for 1 year at 4º C, DO NOT FREEZE

The MPG® mRNA Purification Kit is designed specifically for the isolation of mRNA directly from purified total RNA. The isolation takes 15 minutes or less and eliminates the need to use organic solvents or oligo dT cellulose columns.

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

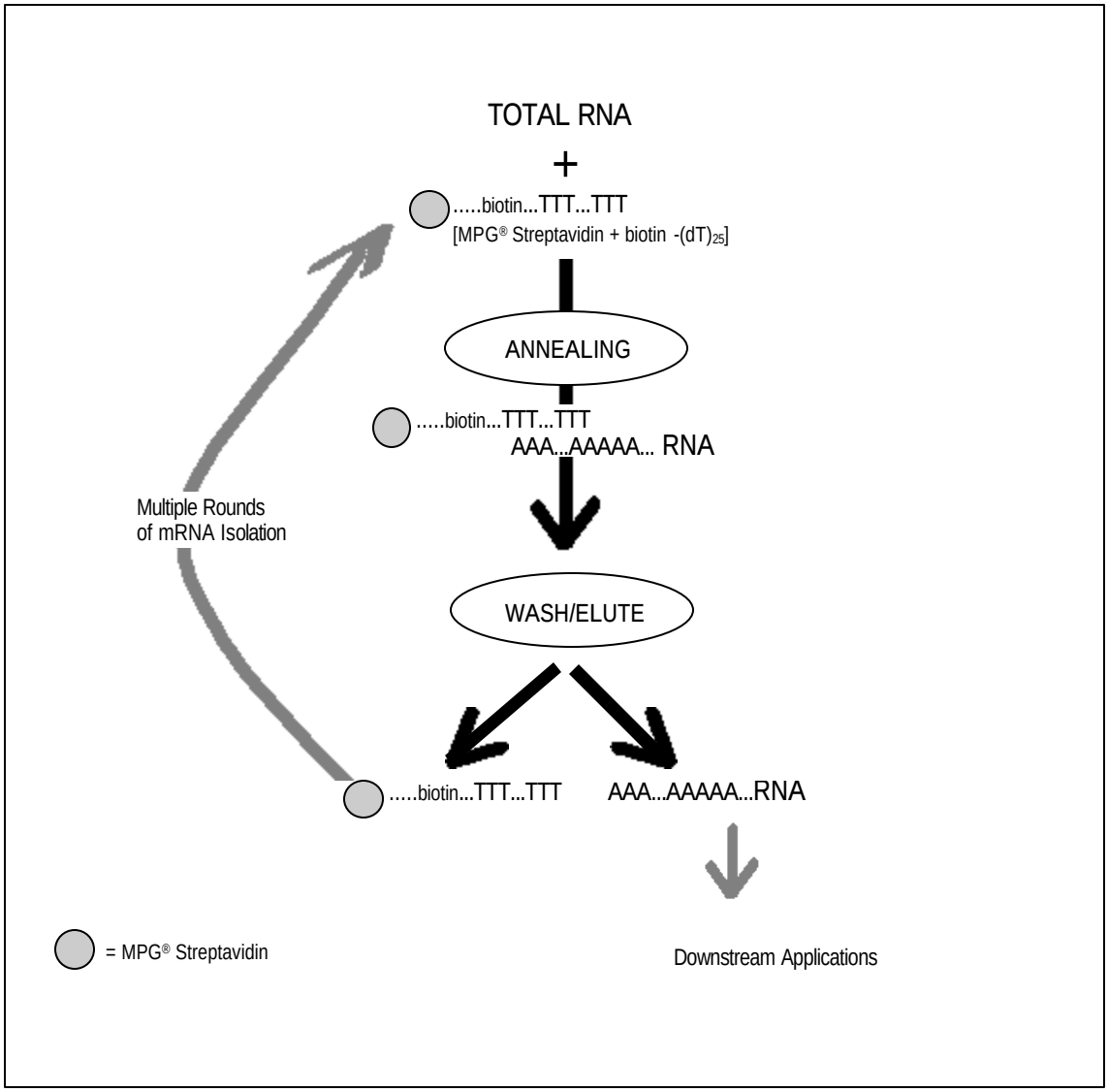
- cDNA Library Construction
- Subtractive Hybridization
- S1 Nuclease Analysis
- Dot Blot Hybridization
- Cloning
- Northern Blotting
- *in vitro* Translation
- Sequencing
- RNA Binding Protein Isolation
- RT-PCR

The MPG® 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 micrometer diameter, 50 nanometer (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® mRNA Purification Kit contains all the reagents necessary to isolate and purify from 30 – 70 µg of high quality mRNA (using mouse liver total RNA) from a single round of isolation.

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)
5 ml	2 X Hybridization Binding Buffer
15 ml	Hybridization Wash Buffer
1 ml	Release Solution

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



Note: *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.*

General Procedure

Materials: (Needed but not supplied)

Total RNA of Interest
Magnetic Particle Separator, Product No. MPS0301 or MPS0001
65°C Water Bath
UV/Vis Spectrophotometer
Nuclease-free Purified Water
1.5 ml Nuclease-free Microcentrifuge Tubes
Vortex Mixer
Nuclease-free Pipettes and Pipette Tips
Low Speed Rotator

*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. [NOTE: THE PROPORTION OF mRNA TO TOTAL RNA VARIES WIDELY IN DIFFERENT TISSUES. mRNA CONSTITUTES ABOUT 2% OF TOTAL RNA ISOLATED FROM MOUSE LIVER. THEREFORE; TO ISOLATE 5 µg OF mRNA, 250 µg OF TOTAL RNA (ISOLATED FROM MOUSE LIVER) PER 1 mg OF MPG® STREPTAVIDIN COMPLEX IS SUGGESTED].

THIS PROTOCOL CAN BE SCALED UP OR DOWN BY PROPORTIONALLY ADJUSTING THE COMPONENT VOLUMES.

Isolation of mRNA from Total RNA

1. Warm the Kit components to room temperature.

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 2 X Hybridization Binding Buffer (350 µl per mg MPG® Streptavidin Complex) and put it aside until ready for hybridization.

4. Add 250 µg of total RNA to a clean nuclease-free microcentrifuge tube. Bring the total volume to 350 µl with nuclease-free water. The final concentration of the total RNA should not exceed 0.75 µg/µl.

5. Disrupt the secondary structure of the total RNA by heating at 65°C for 2-3 minutes.

6. Transfer the heat disrupted total RNA to the tube containing the MPG® Streptavidin Complex. Vortex and incubate 1-3 minutes at room temperature on a low speed rotator. Magnetically separate and carefully remove the supernatant.

7. Resuspend the mRNA-bound MPG® Streptavidin Complex in Hybridization Wash Buffer (350 µl per mg MPG® Streptavidin Complex). Magnetically separate and carefully remove the supernatant. Repeat two more times.

8. 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.

9. The MPG® Streptavidin Complex may be used repeatedly for multiple rounds of isolation from the same total RNA. Resuspend the particles in 2 X Hybridization Binding Buffer and follow Steps 3 through 7. The resulting supernatants may be pooled.

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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