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Products for Biotechnology

With Magnetic Porous Glass (MPG®)

Protocol No.: 5.607
Product: MPG® Streptavidin (10 mg/ml, 4-6 × 10^7 particles/ml)
Procedure: Isolation and purification of mRNA from Total RNA
Storage: 4°C - DO NOT FREEZE

PRODUCT NUMBER	DESCRIPTION	VOLUME
MSTR0502	MPG® Streptavidin, 5 µm, 50 nm (500 Å) pore diameter	2 ml (20 mg)
MSTR0510		10 ml (100 mg)

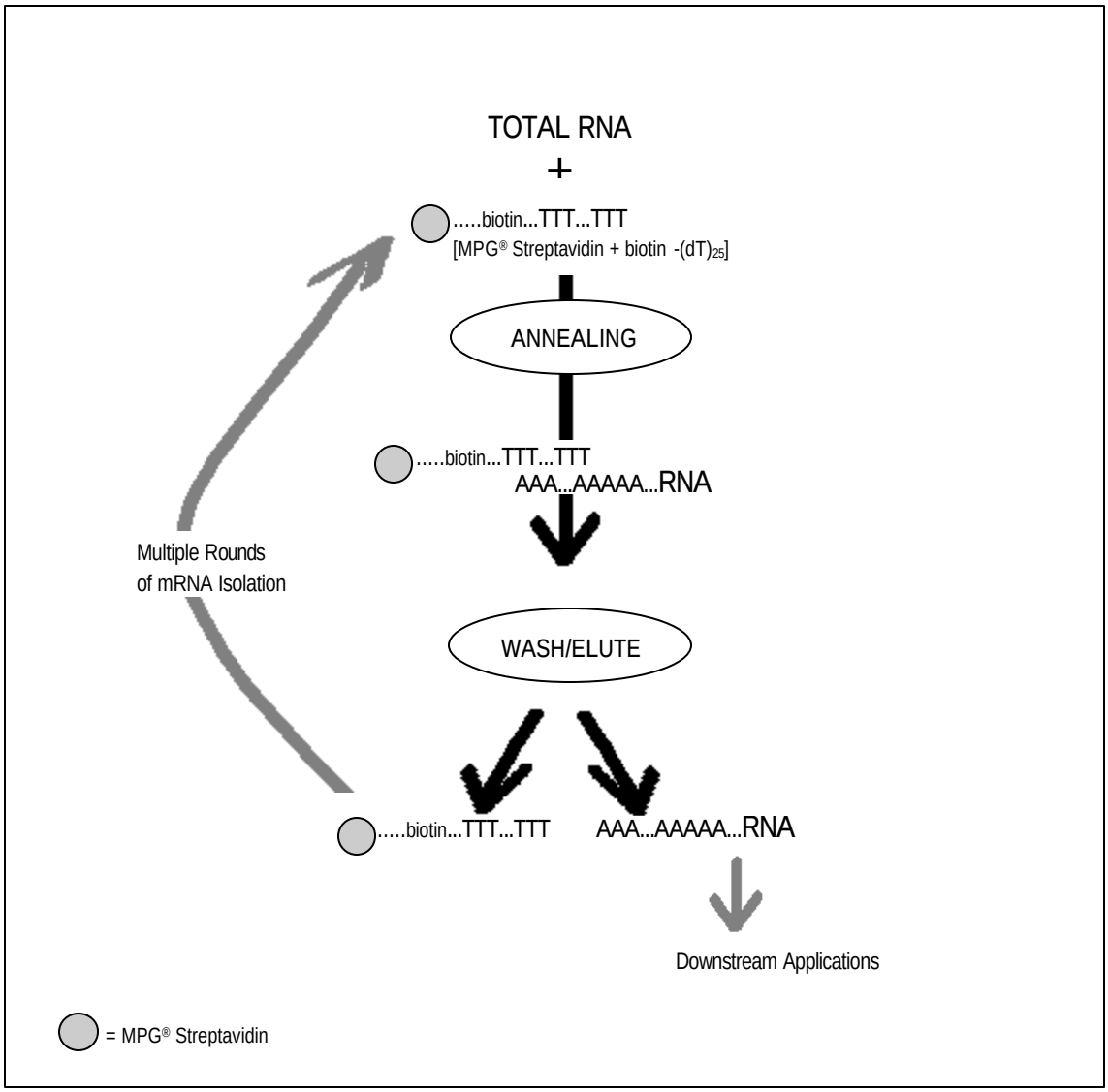
General Procedure

Materials: (based on 1 mg of MPG® Streptavidin, suspended in PBS, pH 7.5, 0.1% BSA, 0.02% NaN_3)

Total RNA of Interest
Biotinylated Oligo $(dT)_{25}$ Probe, Prod. No. MBOLG01
Sterile, Nuclease-free, Deionized Water (dH_2O)
or DEPC-treated water
Potassium Chloride (KCl)
Tris-Base (Tris)
Sodium Chloride (NaCl)
Lithium Chloride (LiCl)
EDTA, disodium ($C_{10}H_{14}N_2O_8Na_2$)
Low Speed end-over-end Rotator
UV/Vis Spectrophotometer
Magnetic Particle Separator, Prod. No. MPS0301 or MPS0001
1.5 ml Nuclease-free Microcentrifuge Tubes
65°C Water Bath
Nuclease-free Pipettes and Pipette Tips
Vortex Mixer

Solution	Preparation
Probe Binding Buffer (1 M KCl, pH 8.0)	Dissolve 7.45 g KCl in 80 ml dH_2O. Adjust to pH 8.0 and bring volume to 100 ml with dH_2O.
Probe Wash Buffer (2 M NaCl, pH 8.0)	Dissolve 11.69 g NaCl in 80 ml dH_2O. Adjust to pH 8.0 and bring volume to 100 ml with dH_2O.
2 X Hybridization Binding Buffer (200mM Tris pH 8.0, 1 M LiCl, 20 mM EDTA)	Dissolve 3.16 g Tris, 4.24 g LiCl and 744 mg EDTA in 80 ml dH_2O. Adjust to pH 8.0 and bring volume to 100 ml with dH_2O.
Hybridization Wash Buffer (10 mM Tris pH 8.0, 150 mM LiCl, 1 mM EDTA)	Dissolve 158 mg Tris, 636 mg LiCl and 37 mg EDTA in 80 ml dH_2O. Adjust to pH 8.0 and bring volume to 100 ml with dH_2O.
Release Solution (0.1 mM EDTA, pH 8.0)	Dissolve 7.5 mg EDTA in 160 ml dH_2O. Adjust to pH 8.0 and bring volume to 200 ml with dH_2O.

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.*

*A REMINDER BEFORE YOU START YOUR PROCEDURE, THIS PROTOCOL IS BASED ON USING 1 mg MPG® STREPTAVIDIN. 1 mg of MPG® STREPTAVIDIN CAN BIND AN AVERAGE OF 5 µg OF mRNA. [NOTE: THE PROPORTION OF mRNA TO TOTAL RNA VARIES WIDELY IN DIFFERENT TISSUES. mRNA CONSTITUTES LESS THAN 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 MILLIGRAM OF MPG® STREPTAVIDIN IS SUGGESTED].

THIS PROTOCOL CAN BE SCALED UP OR DOWN BY PROPORTIONALLY ADJUSTING THE COMPONENT VOLUMES. PER 1 mg OF MPG® STREPTAVIDIN, USE: 800 pmole (10 µl) OF BIOTINYLATED OLIGO $(dT)_{25}$ PROBE AND 90 µl OF PROBE BINDING BUFFER.

Preparation of MPG® Streptavidin Oligo $(dT)_{25}$

1. Warm the solutions to room temperature.

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

3. Resuspend the particles in 100 µl Probe Binding Buffer. Magnetically separate and carefully remove the supernatant. Repeat two more times.

4. Add 800 pmole (10 µl) of Biotinylated Oligo $(dT)_{25}$ Probe and 90 µl Probe Binding Buffer per 1 mg of MPG® Streptavidin used. Vortex well and mix on a rotator for 5 minutes at room temperature. Magnetically separate and carefully remove the supernatant.

5. Resuspend the prepared Oligo $(dT)_{25}$-bound MPG® Streptavidin particles in Probe Wash Buffer (100 µl per 1 mg MPG® Streptavidin) and vortex. Magnetically separate and carefully remove the supernatant. Repeat this step two more times.

6. Resuspend the Oligo $(dT)_{25}$-bound MPG® Streptavidin particles in 2 X Hybridization Buffer (350 µl per mg MPG® Streptavidin) and put it aside until ready for hybridization.

Isolation of mRNA from Total RNA

1. 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.

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

3. Transfer the heat disrupted total RNA to the prepared MPG® Streptavidin-oligo $(dT)_{25}$ particles (from *Preparation of MPG® Streptavidin Oligo $(dT)_{25}$* Section, Step 6). Vortex and incubate 3 minutes at room temperature on a low speed rotator. Magnetically separate and carefully remove the supernatant.

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

5. Resuspend the mRNA-bound MPG® Streptavidin particles in Release Solution (20 µl per mg MPG® Streptavidin) 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.

6. The MPG® Streptavidin-oligo $(dT)_{25}$ 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 1 through 5 in *Isolation of mRNA from Total RNA* Section. 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: We recommended TE Buffer to read OD. Do not use DEPC treated water to read OD, it may lower the A_{260}/A_{280} ratio by 0.2 - 0.3 OD.)

Yield of mRNA (µg/ml)=(OD_{260})(44) (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 -70ºC. Avoid freeze-thaw cycles.

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