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Products for Biotechnology

With Magnetic Porous Glass (MPG®)

Protocol No.: **5.509**
Product: **MPG® Streptavidin** (10 mg/ml, 4-6 × 10^7 particles/ml)
Procedure: **Isolation and purification of mRNA directly from cells, animal or plant tissue using *Guanidine Thiocyanate Homogenization Buffer.***
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)

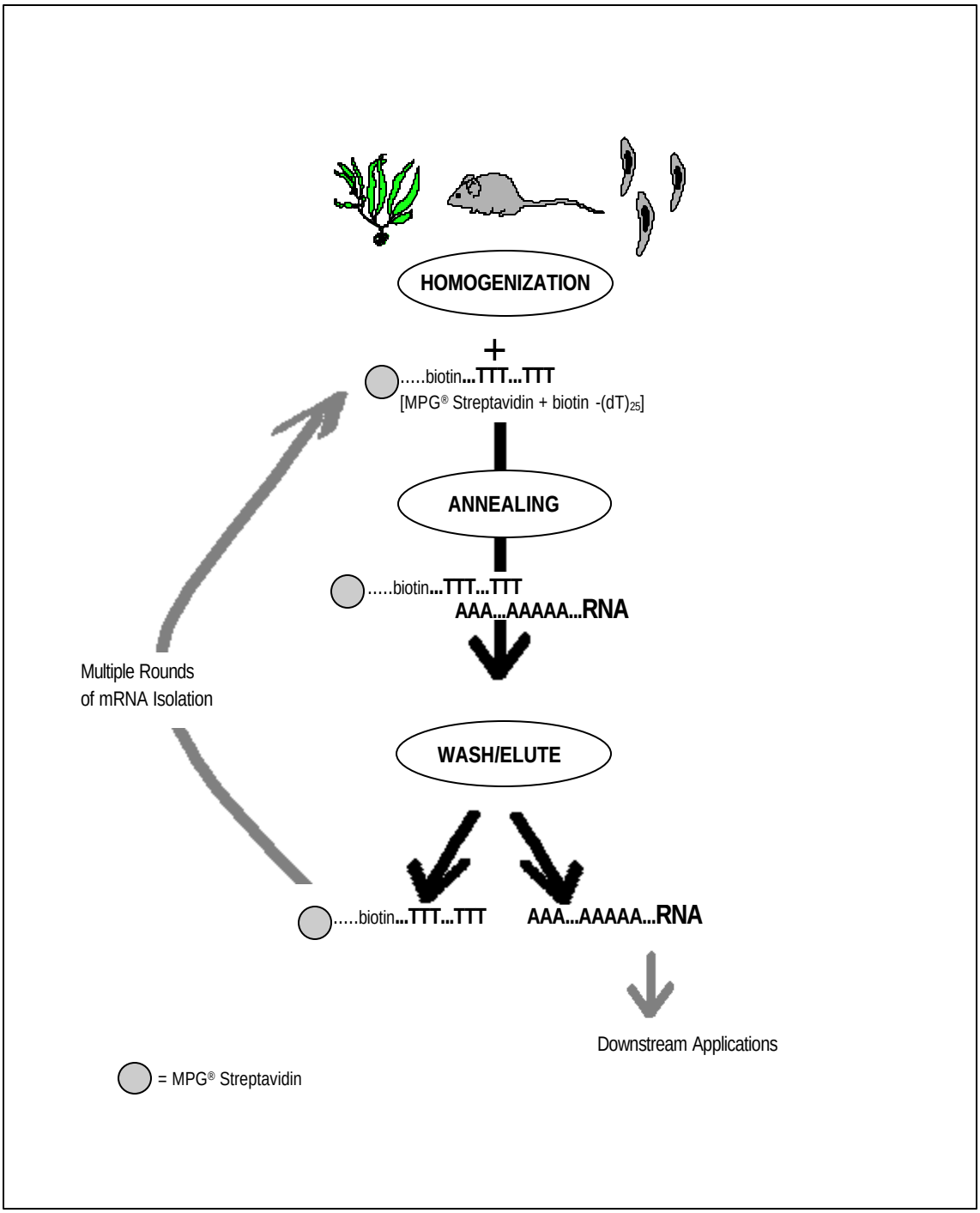
General Procedure

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

Tissue of Interest
Biotinylated Oligo $(dT)_{25}$ Probe, Prod. No. MBOLG01
Sodium, Lauroylsarcosinate ($C_{15}H_{28}NO_3Na$)
Guanidine Thiocyanate ($CH_5N_3 \cdot HSCN$,GTC)
Sterile, Nuclease-free, Deionized Water (dH_2O)
or DEPC treated water
Potassium Chloride (KCl)
Tris-Base (Tris)
Sodium Chloride (NaCl)
Lithium Chloride (LiCl)
ß -Mercaptoethanol ($HSCH_2CH_2OH$)
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
15 ml Sterile Screw Cap Conical Tubes
Microcentrifuge
Mini-Homogenizer or Polytron
65°C Water Bath
Nuclease-free Pipettes and Pipette Tips
Vortex Mixer
Filter (0.2 µm cellulose nitrate membrane)

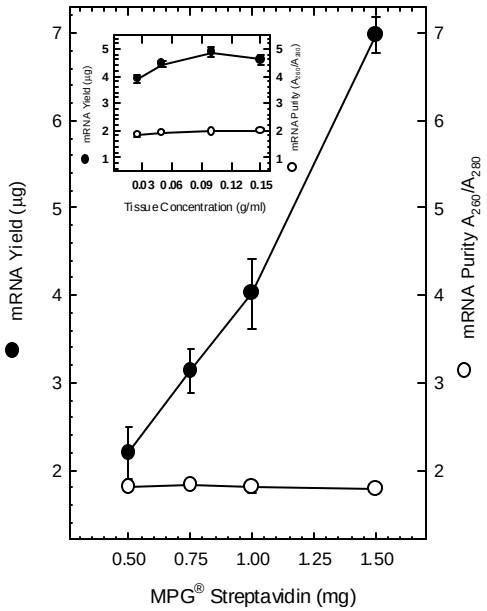
Solution	Preparation
Lauroylsarcosinate (25% stock solution)	Dissolve 2.5 g Lauroylsarcosinate in 8 ml dH_2O. Bring volume to 10 ml with dH_2O.
GTC Homogenization Buffer (4.0 M Guanidine Thiocyanate, 0.5% Lauroylsarcosinate, 100 mM Tris, pH 7.2)	Dissolve 50 g of Guanidine Thiocyanate in 50 ml of 0.2 M Tris pH 7.2. Add 2.0 ml Lauroylsarcosinate 25% stock solution (final concentration 0.5%) and bring volume to 100 ml with dH_2O. Filter the solution through a 0.2 µm cellulose nitrate membrane. Store at room temperature.
GTC Solution	Transfer 1.5 ml of GTC Homogenization Buffer into a 15 ml conical tube. Add 15 µl of ß-Mercaptoethanol. Do this just prior to each use. Chill this tube on ice.
Hybridization Binding Buffer (100 mM Tris pH 7.2, 400 mM LiCl, 20 mM EDTA)	Dissolve 1.58 g Tris, 1.7 g LiCl, 744 mg EDTA in 80 ml dH_2O. Adjust to pH 8.0 to dissolve EDTA, then lower to pH 7.2. Bring the volume to 100 ml with dH_2O.
Hybridization Wash Buffer 2 (10 mM Tris pH 8.0, 150 mM LiCl, 1 mM EDTA, 0.1% Lauroylsarcosinate)	Dissolve 158 mg Tris, 636 mg LiCl, 37 mg EDTA in 80 ml dH_2O. Adjust to pH 8.0. Add 0.4 ml Lauroylsarcosinate 25% stock solution (final concentration 0.1%) 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.
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.



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.

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. 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-oligo $(dT)_{25}$. 4 µg mRNA was isolated per mg of MPG® Streptavidin. 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. 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. 1 mg of MPG® STREPTAVIDIN CAN BIND UP TO 5 µg OF mRNA. 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 PROBE BINDING BUFFER. OPTIMAL RESULTS WILL BE OBTAINED USING FRESH CELLS OR TISSUE.

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

1. Warm the solutions to room temperature except the GTC Homogenization Buffer.

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 of 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 Hybridization Binding Buffer (100 µl per 1 mg MPG® Streptavidin) and put it aside until ready for hybridization.

Isolation of Tissue/Cells

1. Prepare GTC Solution. (See page 1 Solution/Preparation.) 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. Homogenize for 1-2 minutes. (For cultured cells, lyse the cells directly in GTC Solution). Keep on ice. Alternatively, for isolation of mRNA from plant tissue use 0.1-0.2g/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. Mix well. Transfer to microcentrifuge tubes and centrifuge for 45 seconds to 1.5 minutes at 14,000 x g.

Direct Isolation of mRNA

1. Carefully remove the supernatant from the prepared Oligo $(dT)_{25}$-bound MPG® Streptavidin particles (from *Preparation of MPG® Streptavidin Oligo $(dT)_{25}$* Section, Step 6) and add 1.0 - 1.5 ml tissue supernatant per milligram of the prepared Oligo $(dT)_{25}$ -bound MPG® Streptavidin particles. Vortex well and incubate with gentle mixing for 5 minutes at room temperature. Magnetically separate and carefully remove the supernatant.

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

3. 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. 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: We recommend the use of 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 -70°C. Avoid freeze-thaw cycles.

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