752A Lincoln Blvd.
Middlesex, NJ 08846

Toll Free: 866-252-7771
Phone: 732-469-7771 Fax: 732-469-7782
Web: www.purebiotechllc.com

Products for Biotechnology

With Magnetic Porous Glass (MPG®)

Protocol No.: 66.204
Product: MPG® Streptavidin Biotinylated Oligo $(dT)_{25}$ Complex (10 mg/ml, 4-6 × 10^7 particles/ml)
Procedure: Isolation and purification of mRNA directly from cells, animal or plant tissue.
Storage: 4°C - DO NOT FREEZE

PRODUCT NUMBER	DESCRIPTION	VOLUME
MSTR0502C	MPG® Streptavidin Biotinylated Oligo $(dT)_{25}$ Complex 5 µm, 50 nm (500 Å) pore diameter	2 ml (20 mg)

General Procedure

Isolation of mRNA from cells or animal tissue containing high levels of RNase may require Guanidine Thiocyanate Homogenization Buffer. Protocol 66.3 *Isolation and Purification of mRNA Directly from Cells or Animal Tissue using Guanidine Thiocyanate Homogenization Buffer* should be followed.

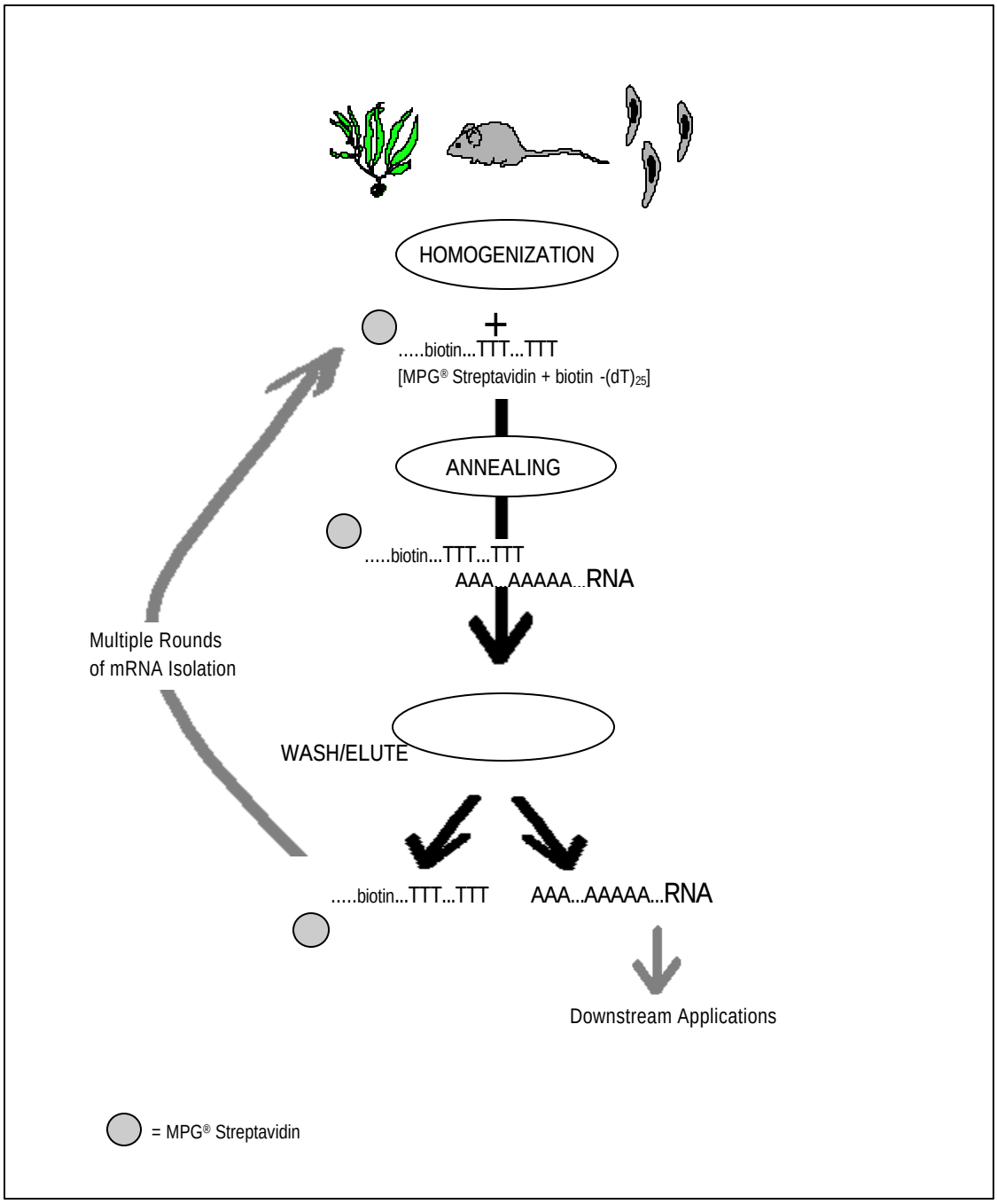
Materials: MPG® Streptavidin Biotinylated Oligo $(dT)_{25}$ Complex
(10 mg, suspended in 50 mM Tris HCl, pH 7.2, 2.0 M NaCl, 0.02% NaN_3)

Tissue of Interest
Lithium Dodecyl Sulfate ($C_{12}H_{25}O_4SLi$, LiDS)
Sterile, Nuclease-free, Deionized Water (dH_2O)

or DEPC-Treated Water
Tris-Base (Tris)
Lithium Chloride (LiCl)
Dithiothreitol ($C_4H_{10}O_2S_2$, DTT)
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
50 ml Sterile Screw Cap Conical Tubes
Microcentrifuge
Mini-Homogenizer or Polytron
65°C Water Bath
Nuclease-free Pipettes and Pipette Tips
Vortex Mixer

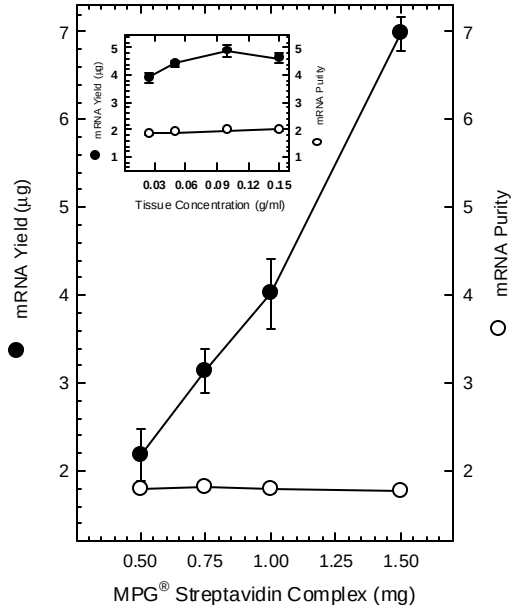
Solution	Preparation
Tissue Extraction/Hybridization Buffer (100 mM Tris, 500 mM LiCl, 10 mM EDTA, 1% LiDS, 5 mM DTT, pH 8.0)	Dissolve 1.58 g Tris, 2.12 g LiCl, 372 mg EDTA, 100 mg LiDS and 77 mg DTT in 80 ml dH_2O. Adjust to pH 8.0 and bring volume to 100 ml with dH_2O. (Shelf life: 1 week at 4°C)
Hybridization Wash Buffer 1 (10 mM Tris, 150 mM LiCl, 1 mM EDTA, pH 8.0)	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.
Hybridization Wash Buffer with LiDS (10 mM Tris, 150 mM LiCl, 1 mM EDTA, 0.1% LiDS, pH 8.0)	Dissolve 158 mg Tris, 636 mg LiCl, 37 mg EDTA and 100 mg LiDS 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 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 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.*

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*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 Buffer .

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 Buffer (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 106-107 cells per ml). Homogenize for 1-2 minutes on ice. Alternatively, 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 low temperature ice bath (liquid nitrogen or acetone - dry ice).

2. Transfer homogenate to 1.5 ml nuclease-free microcentrifuge tube(s) and 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 1. 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 may lower the A_{260}/A_{280} ratio by 0.2 - 0.3 OD.) It is recommended to use UV-transparent quartz cuvettes.

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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