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# Products for Biotechnology

**With Magnetic Porous Glass (MPG®)**

**Protocol No.:** **66.303**
**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 using *Guanidine Thiocyanate Homogenization Buffer.***
**Storage:** **4°C - DO NOT FREEZE**

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

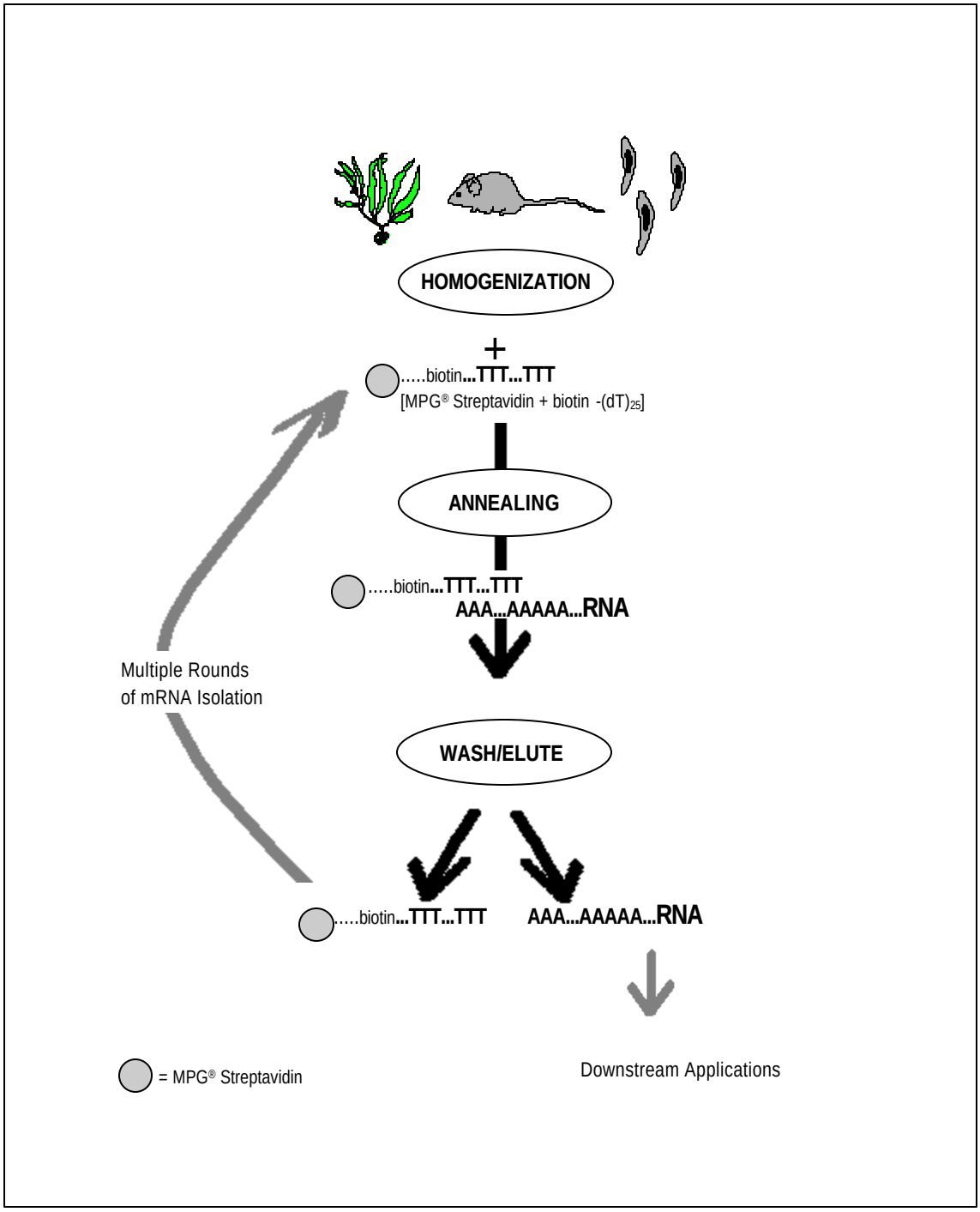
## General Procedure

**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
Sodium, Lauroylsarcosinate ($C_{15}H_{28}NO_3Na$)
Guanidine Thiocyanate ($CH_5N_3$·HSCN,GTC)
Sterile, Nuclease-free, Deionized Water ($dH_2O$)
or DEPC treated water
Tris-Base (Tris)
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
50 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)

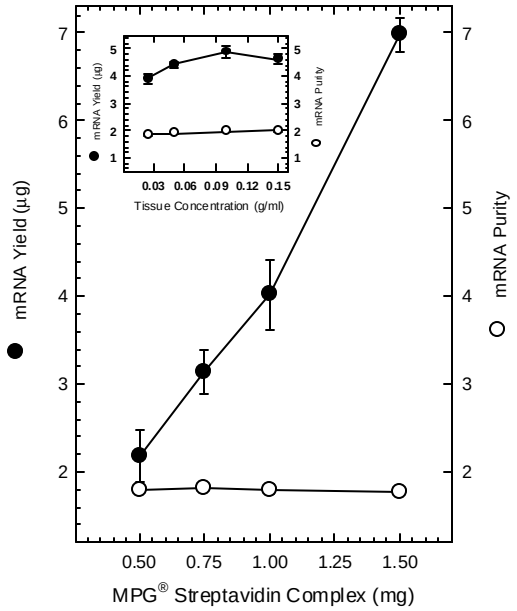
| Solution | Preparation |
|---|---|
| Lauroylsarcosinate (25% stock solution) | Dissolve 2.5 g Lauroylsarcosinate in 8 ml $dH_2O$. Bring volume to 10 ml with $dH_2O$. |
| GTC Homogenization Buffer (4.0 M Guanidine Thiocyanate, 0.5% Lauroylsarcosinate, 100 mM Tris, pH 7.2) | Dissolve 50 g of Guanidine 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 50 ml centrifuge 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 disso 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 $dH_2O$. |




***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 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 (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*.

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

**Preparation of Tissue/Cells**

1. Prepare GTC Solution. (See page 1 Solution/Prepartion.) 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 from step 1 above. 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 0.1-0.2 g/ml and grind the plant tissue using a mortar and pestle on a low temperature ice bath (liquid nitrogen or acetone - dry ice).

3. Dilute the homogenate with 2 volumes of Hybridization Binding Buffer, (i.e., 3 ml for each 1.5 ml homogenate). Mix well. Centrifuge for 45 seconds to 1.5 minutes at 14,000 x g.

**Direct Isolation of mRNA**

1. 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 MPG® Streptavidin Complex. Vortex well and incubate with gentle 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 2 (1 ml per mg MPG® Streptavidin Complex). Magnetically separate and carefully remove the supernatant. Repeat two more times.

3. 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)

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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*For in vitro research use only.*