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Products for Biotechnology

With Magnetic Porous Glass (MPG®)

Protocol No.: 5.105
Product: MPG® Streptavidin (10 mg/ml, 4-6 × 10^7 particles/ml)
Procedure: Purification of Nucleic Acids
Storage: Refrigerate

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 1.0 mg of MPG® Streptavidin, suspended in PBS, pH 7.5, 0.1% BSA, 0.02% NaN_3)

- DNA of Interest
- Sodium Chloride (NaCl)
- Potassium Chloride (KCl)
- Sodium Hydroxide (NaOH)
- Sterile, Nuclease-Free, Deionized Water (dH_2O)
- Tris Hydrochloride (Tris-HCl)
- Biotinylated Complementary DNA Probe
- EDTA, disodium ($C_{10}H_{14}N_2O_8Na_2$)
- Low Speed Rotator
- 70°C Water Bath
- 1.5 ml Nuclease-free Microcentrifuge Tubes
- Nuclease-free Pipettes and Pipette Tips
- Magnetic Particle Separator, Prod. No. MPS0301 or MPS0001
- Vortex Mixer

Solution	Preparation
2X Binding Buffer: (2 M KCl)	Dissolve 15 g KCl in 80 ml dH_2O. Adjust to pH 7.5 and bring volume to 100 ml with dH_2O.
Washing Buffer (2 M NaCl)	Dissolve 11.6 g NaCl in 80 ml dH_2O. Adjust to pH 7.5 and bring volume to 100 ml with dH_2O.
2X Hybridization Buffer (1 M NaCl)	Dissolve 5.8 g NaCl in 80 ml dH_2O. Adjust to pH 7.5 and bring volume to 100 ml with dH_2O.
Storage Buffer (TE Buffer)	Dissolve 158 mg Tris-HCl and 37.2 mg EDTA in 95 ml of dH_2O. Adjust to pH 7.5 with NaOH and bring volume to 100 ml with dH_2O.

Preparation of MPG® Streptavidin Complementary DNA Probe

1. Transfer 100 µl (1.0 mg) of MPG® Streptavidin into a 1.5 ml nuclease-free microcentrifuge tube. Magnetically separate the MPG® Streptavidin from the solution by placing the tube in a Magnetic Particle Separator for at least 30 seconds. Carefully remove the supernatant with a pipette while the tube remains in the magnetic particle separator.

2. Add 100 µl of 2X Binding Buffer and vortex to mix. Magnetically separate and carefully remove the supernatant.

3. In a separate tube combine 500 pmol Biotinylated Complementary DNA Probe, 50 µl 2X Binding Buffer and dH_2O to 100 µl total volume. Add this mixture to the MPG® Streptavidin. Vortex well and mix for 3 to 5 minutes at room temperature. Magnetically separate and carefully remove the supernatant.

4. Add 100 µl of Washing Buffer to the Complementary DNA probe-bound MPG® Streptavidin particles and vortex to mix. Magnetically separate and carefully remove the supernatant.

Hybridization of DNA

1. In a separate tube, combine 50 µl 2X Hybridization Buffer, DNA of Interest and dH_2O to 100 µl total volume. Place the tube in a 70°C water bath for 2 to 3 minutes to denature the secondary structure. Add this mixture to the Complementary DNA Probe-bound MPG® Streptavidin particles and incubate 5 minutes at room temperature. Magnetically separate and carefully remove the supernatant.

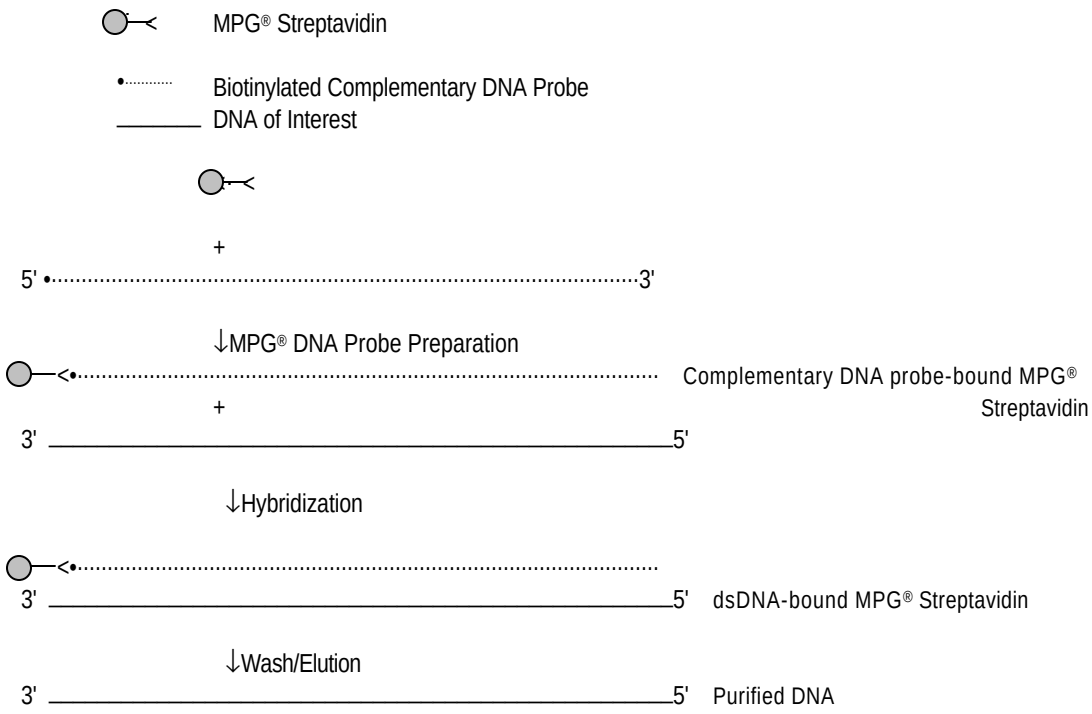
2. Add 100 µl of Washing Buffer and mix. Magnetically separate and carefully remove the supernatant.

Recovery of Hybridized DNA

1. Add 100 µl of dH_2O or Storage Buffer to the dsDNA-bound MPG® Streptavidin particles. Place in a 70°C water bath for 2 to 3 minutes to release the purified ssDNA of Interest. Magnetically separate and quickly, but carefully, transfer the released ssDNA in the supernatant to a second tube. Repeat one more time. The ssDNA is now ready for further manipulation.

2. The ssDNA may be stored dry, frozen at -20°C in Storage Buffer or in ethanol at -20°C.

Schematic Diagram Detailing Purification of Nucleic Acids



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