PUREBIOTECH LLC
752A Lincoln Blvd.
Middlesex, NJ 08846

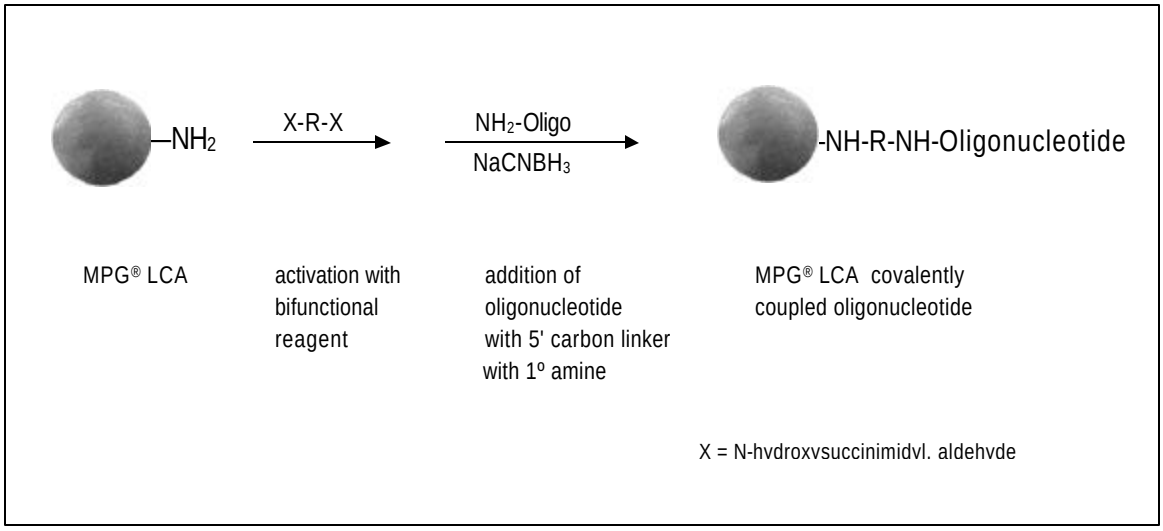
Toll Free: 866-252-7771
Phone: 732-469-7771 Fax: 732-469-7782
Web: www.purebiotechllc.com

Products for Biotechnology

With Magnetic Porous Glass (MPG®)

Protocol No.: 3.206
Product: MPG® Long Chain Alkylamine (30 mg/ml, 1.2 - 1.8 × 10^8 particles/ml)
Procedure: Covalent Attachment of Oligonucleotides.
Storage: Ambient Temperature

PRODUCT NUMBER	DESCRIPTION	VOLUME
MLCA0502	MPG® LCA, 5 µm, 50 nm (500 Å) pore diameter	2 ml (60 mg)
MLCA0510		10 ml (300 mg)



General Procedure

Materials: (Based on 10 mg MPG® Long Chain Alkylamine, suspended in 10 mM phosphate, pH 7.5, 0.15 M NaCl)

Oligonucleotide with a modified 5' end containing a 6 carbon extension ending with a primary amine
Sodium Cyanoborohydride ($NaBH_3CN$)
Sodium Phosphate, Monobasic (NaH_2PO_4)
Sodium Phosphate, Dibasic, Heptahydrate(Na_2HPO_4)
Sodium Azide (NaN_3)
Deionized Water (dH_2O)
Glycine (H_2NCH_2COOH)
Sodium Chloride (NaCl)
25% Glutaraldehyde ($CHO(CH_2)_3CHO$)
2N Hydrochloric Acid (HCl)
Vortex Mixer
Low Speed Rotator
1.5 ml Microcentrifuge Tubes
Pipette and Pipette Tips
Magnetic Particle Separator, Prod. No. MPS0301 or MPS0001

Solution	Preparation
Coupling Buffer (10 mM Phosphate, pH 7.5)	Dissolve 19.2 mg NaH_2PO_4 and 225.2 mg $Na_2HPO_4 \cdot 7H_2O$ in 80 ml dH_2O. Adjust to pH 7.5 with 2N HCl, if necessary, and bring volume to 100 ml with dH_2O.
Activation Solution (12.5% Glutaraldehyde)	Add 0.4 ml 50% Glutaraldehyde to 1.2 ml Coupling Buffer (make fresh for each reaction).
1% Sodium Cyanoborohydride Solution (Fresh)	Dissolve 10 mg $NaBH_3CN$ in 1 ml Coupling Buffer.
1% Glycine Solution	Dissolve 10 mg Glycine in 1 ml Coupling Buffer.
Wash Buffer (10 mM Phosphate, pH 7.5, 1.0 M NaCl)	Dissolve 584.7 mg NaCl in 8 ml of Coupling Buffer. Bring to 10 ml with Coupling Buffer.
Storage Buffer (10 mM Phosphate, pH 7.5, 150 mM NaCl, 0.02% NaN_3)	Dissolve 87.7 mg NaCl, and 2 mg NaN_3 in 8 ml of Coupling Buffer. Bring to 10 ml with Coupling Buffer.

Activation of MPG® Long Chain Alkylamine

1. Adjust the concentration of MPG® Long Chain Alkylamine to 10 mg/ml. Transfer 1 ml to a 1.5 ml microcentrifuge tube. Magnetically separate the MPG® Long Chain Alkylamine from the solution by placing the tube in a Magnetic Particle Separator for at least 30 seconds. Remove the supernatant by aspiration while the tube remains in the particle separator.

2. Add 1 ml of Coupling Buffer and mix well. Magnetically separate and aspirate the supernatant.

3. Add 250 µl of Activation Solution to the MPG® Long Chain Alkylamine particles, mix well and place in a low speed rotator for 1½ hours at room temperature. Magnetically separate and aspirate the supernatant.

4. Add 1 ml of Coupling Buffer to the activated MPG® Long Chain Alkylamine particles and mix well. Magnetically separate and remove the supernatant. Repeat this step four more times.

Coupling of Oligonucleotide to Activated MPG® Long Chain Alkylamine

1. Dissolve 1 mg oligonucleotide in 1 ml of Coupling Buffer. Add this mixture and 100 µl of 1% Sodium Cyanoborohydride Solution to the activated MPG® Long Chain Alkylamine particles. Mix well and rotate 5-8 hours at room temperature. Magnetically separate and aspirate the supernatant.

2. Add 1 ml of 1% Glycine Solution and 100 µl of 1% Sodium Cyanoborohydride Solution, mix well and rotate 2 hours at room temperature. Magnetically separate and aspirate the supernatant.

3. Add 1 ml of Washing Buffer and mix well. Magnetically separate and remove the supernatant. Repeat this step five more times. The oligonucleotide-bound MPG® Long Chain Alkylamine is ready to use.

4. For storage, add 1 ml of Storage Buffer to the oligonucleotide-bound MPG® Long Chain Alkylamine and mix well. Magnetically separate and aspirate the supernatant. Resuspend the oligonucleotide-bound MPG® Long Chain Alkylamine particles in 1 ml Storage Buffer and store at 4°C.

This is a modification of protocol 3.1, *Covalent Attachment of Proteins*, as described by Eberwine, J. 1996 Biotechniques 20:584-591.

FOR TECHNICAL SERVICE ON THIS OR ANY OTHER PureBiotech PRODUCT CALL 866-252-7771
or e-mail us at info@purebiotechllc.com.

For in vitro research use only.