

Coupling Procedure for Attaching Oligonucleotides to BioMag[®] Carboxyl

Description

BioMag Carboxyl consists of an aqueous suspension of magnetic iron oxide particles coated to provide carboxyl groups. The carboxyl groups are sterically unencumbered, permitting the covalent attachment of oligonucleotides with retention of biological activity for molecular biology applications. There are about 4.8 μ moles of carboxyl per ml (240 μ moles per gram).

Characteristics

Mean Diameter: ~1.5 μ m
Concentration: 20mg/ml

Material

Material Required

- BioMag Carboxyl (Cat. #84125) or BioMagPlus Carboxyl (Cat. #86011)
- Amine-terminated oligonucleotide to be attached to the BioMag: 10mg
- Carbodiimide (EDAC): 384mg
- 0.1 M Imidazole Buffer (pH 7.0): Add 8.22ml of 1-Methylimidazole to 900ml of DEPC-treated deionized water. QS to 1L with DEPC-treated deionized water and adjust to pH 7.0 as necessary. *Note:* The DEPC-treated water must be autoclaved before making the buffer.
- Prehybridization Buffer (pH 7.4): 0.1 M Tris, 0.005 M EDTA, 0.5% N-Laurylsarcosine, 1% Nuclease-free BSA in DEPC-treated deionized water at pH 7.4. *Note:* The DEPC-treated water must be autoclaved before making the buffer.
- DEPC-treated water
- BioMag Flask Separator (Cat. #84101S)
- Rotator

Procedure

Researchers are advised to optimize the use of particles in any application. Researchers are also advised to wear gloves and lab coats whenever handling Molecular Biology reagents.

The following procedure is for the attachment of 10mg of amine terminated oligonucleotide to 200mg of BioMag Carboxyl. This procedure may be modified with regard to scale.

Preparation of BioMag Carboxyl Particles

1. Transfer 10ml (200mg) of BioMag Carboxyl to a sterile reaction flask which will comfortably hold the maximum volume of 40ml used in the coupling procedure. *Note:* A 50ml tissue culture flask or conical tube is typically used.
2. Add 40ml of DEPC-treated water, shake vigorously, and magnetically separate until the supernatant is clear (approximately 10 minutes). *Note:* The reaction flask may be secured to the magnetic flask separator with a rubber band.
3. Aspirate the supernatant, leaving the BioMag as a wet cake on the container wall.
4. Repeat Steps 2-3 one more time.

Preparation of EDAC/Oligonucleotide Solution

1. Add 384mg of EDAC to 20ml of 0.1 M Imidazole Buffer. Rotate until completely dissolved. *Note:* Make the EDAC solution immediately prior to use. Do not make up in advance.

2. Resuspend the amine terminated oligonucleotide in EDAC solution or DEPC-treated water. Make sure that the oligonucleotide is completely dissolved.
3. Transfer 10mg of amine terminated oligonucleotide to the 20ml of EDAC solution from Step 1. Mix well.

Coupling of Oligonucleotide

1. Immediately add the Oligonucleotide/EDAC solution from the previous section to the washed BioMag. Mix well. Rotate 16-24 hours at room temperature.
2. Magnetically separate and aspirate the supernatant. Add 40ml of DEPC-treated water and shake vigorously. Magnetically separate and aspirate the supernatant. Repeat three times.
3. Add 60ml of Prehybridization Buffer. Shake vigorously and incubate at 68°C for 4 hours, shaking vigorously every 30 minutes.
4. At the end of the 4 hour 68°C incubation period, rotate 16-24 hours at room temperature.

Dilution of Coupled BioMag Particles

1. Magnetically separate and aspirate the supernatant.
2. Resuspend the oligonucleotide-linked BioMag in 40ml of Prehybridization Buffer.

Storage and Stability

Store at 4°C. Freezing, drying, or centrifuging particles may result in irreversible aggregation and loss of binding activity.

Safety

Researchers are advised to wear gloves and lab coats whenever handling molecular biology reagents.

This product is for research use only and is not intended for use in humans or for *in vitro* diagnostic use.

Ordering Information

Cat. #	Description	Sizes
84125	BioMag® Carboxyl	10ml or 100ml
86011	BioMag®Plus Carboxyl	10ml
84101S	BioMag® Flask Separator	1 each
84102S	BioMag® 15ml/50ml Tube Separator	1 each

To Order

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