BioMag®Plus Carboxyl & BioMag®Plus Carboxyl Protein Coupling Kit

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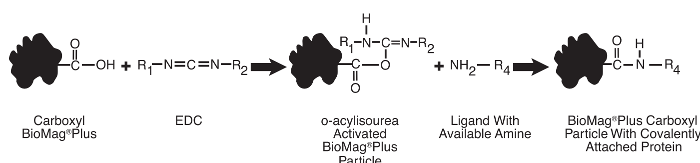
BEADS ABOVE THE REST™

DESCRIPTION

BioMag® and BioMag®Plus superparamagnetic microparticles are utilized in the magnetic separation of cells, organelles, proteins, immunoglobulins, nucleic acids, and many other types of molecules in biological and non-biological systems. The irregular shape of BioMag® and BioMag®Plus affords a much greater surface area than that of the same size spherical particles. This large surface area results in high binding capacities, allowing efficient target capture with conservative use of particles. Additionally, their greater than 90% iron oxide content allows for faster magnetic separations, particularly on automated high throughput platforms.

BioMag® and BioMag®Plus particles are manufactured using the same process, though BioMag®Plus undergo additional processing for the size distribution. Additionally, all kits within this product line feature BioMag®Plus as the principle component.

BioMag®Plus Carboxyl particles offer a high level of carboxyl functionality on magnetically responsive particles. Typically, the carboxyl groups on the surface of the particles are activated by carbodiimide, allowing amine groups on proteins to be covalently attached.



Bangs offers the BioMag®Plus Carboxyl Protein Coupling Kit for covalently coupling proteins to BioMag®Plus superparamagnetic particles. The contents of the kit are sufficient for five coupling reactions. To use the kit for smaller or larger samples, adjust all volumes in a proportional manner.

CHARACTERISTICS

Mean Diameter:	~1.5µm
Particle Concentration:	20 mg/mL

MATERIAL

Material Supplied

- BioMag®Plus Carboxyl: 2.5mL
- EDAC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide): 0.10g
- 15mL conical centrifuge tubes: 5 tubes
- BioMag® MultiSep Magnetic Separator
- 0.05M MES Buffer (pH 5.2): 2 x 175mL
- Quenching Solution (1M Glycine, pH 8.0): 25mL
- Wash Buffer: 125mL

PROCEDURE

Researchers are advised to optimize the use of particles in any application. Activation and Protein Coupling steps should be performed in a well-ventilated chemical fume hood.

Activation

1. Transfer 0.5mL (10mg) of BioMag®Plus Carboxyl particles to a 15mL conical centrifuge tube. Magnetically separate the particles until the supernatant is clear. Aspirate the supernatant and discard.
2. Add 5mL of MES Buffer and shake well to mix. Magnetically separate the particles until the supernatant is clear. Aspirate the supernatant and discard.
3. Repeat Step 2, three times. After the final wash, resuspend the particles in 5mL of MES Buffer.
4. Remove the EDAC from frozen storage and thaw at room temperature for a minimum of 30 minutes. Accurately measure out the required mass of EDAC (1.6mg EDAC/mg BioMag®Plus Carboxyl), and add the EDAC to the particles in solution. Shake vigorously or vortex to mix.
5. Place the flask on a non-magnetic mixing device (rotator) for 30 minutes at room temperature. *Note:* The particles should not be allowed to settle during mixing.
6. Magnetically separate the activated particles until the supernatant is clear. Aspirate the supernatant and discard.
7. Repeat Step 2, four times.

Protein Coupling

1. Calculate the amount of protein, antibody, or other ligand required for coupling. Generally, total protein concentrations of 20-500µg should be used for each milligram of activated BioMag®Plus Carboxyl particles. Carrier proteins, such as Bovine Serum Albumin (BSA) Fraction V, may be added to increase total protein concentration, blocking, and provide proper orientation of specific ligand binding for covalent attachment.
2. Add the protein to a volume of 5mL MES Buffer.
3. Remove 50µL of diluted protein solution and add it to 950µL of MES Buffer. This is a 1:20 dilution. Label as Pre-Coupling Solution. Set aside for Coupling Efficiency Determination.
4. Add the remaining protein solution (oligomer) to the activated particles and shake vigorously or vortex to mix. Place the centrifuge tube on a non-magnetic mixing device for 16-24 hours at room temperature.
5. Magnetically separate the particles until the supernatant is clear, and save. Label as Post-Coupling Solution. Set aside for Coupling Efficiency Determination.
6. Resuspend the particles in 5mL MES Buffer and shake vigorously or vortex to mix. Magnetically separate the particles until the supernatant is clear. Aspirate the supernatant and discard.
7. Repeat Step 6, one time.
8. Add 5mL of Quenching Solution. Shake vigorously or vortex to mix. Place the tube on a non-magnetic mixing device for 30 minutes at room temperature.
9. Magnetically separate the particles until the supernatant is clear. Aspirate the supernatant and discard.

Washing and Diluting Coupled Particles

1. Add 5mL of Wash Buffer and shake vigorously or vortex to mix. Magnetically separate and discard supernatant.
2. Repeat Step 1, three times.
3. After the last wash, resuspend the particles to 2mL in Wash Buffer. Particle concentration is now approximately 5 mg/mL.
4. Store the coupled BioMag®Plus particles at 2-8˚C as a suspension in Wash Buffer.

Coupling Efficiency Determination

1. Set up a UV / Vis spectrophotometer to measure at 280nm. Fill both cuvettes with MES buffer and blank the spectrophotometer.
2. Measure the absorbance of the Pre-Coupling Solution and the Post-Coupling Solution. Note: Further dilutions may be necessary to read the absorbance within the linear range of the instrument.
3. Calculate the coupling efficiency, expressed as the % Protein Uptake, as follows. Typical values of Protein Uptake are >60%.

$$\frac{[(A_{280}\text{ Pre-Coupling Solution x D}) - (A_{280}\text{ Post-Coupling Solution x D})] \text{ x } 100}{(A_{280}\text{ Pre-Coupling Solution x D})}$$

NOTES

1. Avoid the use of amine (e.g. Tris) or carboxyl (e.g. acetate, citrate) buffers in the coupling step. Phosphate is satisfactory in the Coupling Buffer (i.e. prior to the attachment of protein). Buffers containing amine or carboxyl groups can be used as Wash Buffers.
2. Some noncovalent adsorption invariably accompanies covalent coupling to particulate supports. Noncovalent adsorption is controlled by the washing procedure used after covalent protein attachment. The degree of noncovalent adsorption varies with each application and the washing procedure may have to be adjusted for individual applications. Additional washes to reduce noncovalently adsorbed protein can include high salt (1M NaCl), mildly acidic or basic media, mildly elevated temperatures, or increased time of exposure to the Wash Buffer. Dissociation of active, noncovalently adsorbed molecules from BioMag®Plus particles can make magnetic materials appear unstable in some applications.
3. Prolonged vigorous shaking or vortexing should be used to resuspend BioMag®Plus particles after magnetic separation or settling with gravity.

STORAGE AND STABILITY

Store at 2-8˚C. Freezing, drying, or centrifuging particles may result in irreversible aggregation and loss of binding activity.

SAFETY

The suspension as supplied does not contain sodium azide. However, the suggested Wash Buffer does contain NaN_3. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Material Safety Data Sheet for more information.

These products are for research use only and are not intended for use in humans or for *in vitro* diagnostic use.

ORDERING INFORMATION

Cat. Code	Description	Size
BP618	BioMag®Plus Carboxyl	10mL
BP611	BioMag®Plus Carboxyl Protein Coupling Kit	1 kit

Order online anytime at **www.bangslabs.com.**