Product Data Sheet 617

BioMag®Plus Amine & BioMag®Plus Amine Protein Coupling Kit

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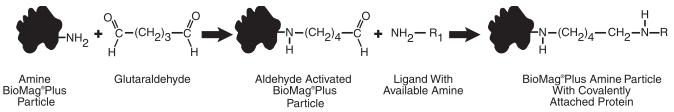
B E A D S ● A B O V E T H E R E S T ™

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 particles 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 minimal amounts of particles. Additionally, their greater than 90% iron oxide content allows for faster magnetic separations, especially on automated high throughput platforms.

BioMag®Plus particles are similar to conventional BioMag® particles with the distinctions of having reduced size distribution and that all BioMag®Plus particles are offered in kits as the principle component.

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



Bangs offers the BioMag®Plus Amine 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:	50 mg/mL

MATERIAL

Material Supplied

Quantity	Kit Component	Volume
1	BioMag®Plus Amine	10mL
1	Glutaraldehyde (EM Grade, 25%) *Note:* Dilute to 5% before use with pyridine wash buffer (PWB).	10mL
5	Conical Centrifuge Tubes	50mL
1	BioMag® MultiSep Magnetic Separator *Note:* Accommodates 50mL, 15mL, and 1.5mL tubes.	-----
1	Pyridine Wash Buffer (PWB), 0.1M *Note:* Dilute 1:10 using deionized water and adjust pH to 6.0 before use.	100mL
1	Quenching Solution (1M Glycine, pH 8.0)	100mL
1	Wash Buffer *Note:* Dilute 1:10 using deionized water and adjust pH to 7.4-7.6 before use.	50mL

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 2mL of BioMag®Plus Amine particles (equivalent to 100mg) to a centrifuge tube.
2. Add 16mL of Pyridine Wash Buffer (PWB) to the tube and shake vigorously or vortex to mix. Magnetically separate the particles until the supernatant is clear. Aspirate the supernatant and discard.
3. Repeat Step 2, three times.
4. If not done so already, dilute 25% glutaraldehyde to 5% with PWB. Add 8mL of 5% glutaraldehyde to the particles and shake vigorously or vortex to mix.
5. Place the tube on a non-magnetic mixing device (rotator) for 3 hours 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. Determine the amount of protein, antibody, or other ligand required for coupling. Generally, total protein concentrations of 50-500µg should be used for each milligram of activated BioMag®Plus Amine 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. Dissolve the protein in 8mL PWB.
3. Remove 20µL of diluted protein solution and add it to 380µL of PWB. This is a 1:20 dilution. If further dilution is necessary, dilute accordingly. Label as Pre-Coupling Solution. Set aside for Coupling Efficiency Determination.
4. Add the remaining protein solution to the activated particles and shake vigorously or vortex to mix. Place the tube on a rotator for 16-24 hours at room temperature.
5. Magnetically separate the coupled particles until the supernatant is clear, and save. If necessary, dilute accordingly. Label as Post-Coupling Solution. Set aside for Coupling Efficiency Determination.
6. Resuspend the particles in 16mL of PWB.
7. Add 8mL of Quenching Solution. Shake vigorously or vortex to mix. Place the tube on a rotator for 30 minutes at room temperature.

8. Magnetically separate the particles until the supernatant is clear. Aspirate the supernatant and discard.

Washing and Diluting Coupled Particles

1. Add 16mL of Wash Buffer and shake vigorously or vortex to mix. Magnetically separate the particles until the supernatant is clear. Aspirate the supernatant and discard.
2. Repeat Step 1, three times.
3. Resuspend the particles to 20mL in Wash Buffer. Particle concentration is now approximately 5 mg/mL. 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 PWB 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%.

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

NOTES

1. Phosphate buffer (0.01M, pH 7.0) can be used as a coupling buffer, but with reduced coupling efficiency compared to the recommended pyridine buffer. The polyvalent, negative phosphate ions clump the positively charged amine support. Do not use primary amines, ammonium ion, or other strong nucleophiles in the coupling buffer. All coupling buffers should be used at minimal ionic strengths. Buffers containing amines (e.g. Tris) or phosphate buffers (e.g. PBS) can be used as Wash Buffers. Ionic strength has little or no effect on BioMag®Plus particles once protein is attached.
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
BP617	BioMag®Plus Amine	10mL
BP610	BioMag®Plus Amine Protein Coupling Kit	1 kit

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