Product Data Sheet 640

BioMag® Maxi Amine

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B E A D S ● A B O V E T H E R E S T ™

DESCRIPTION

BioMag® Maxi Amine is an aqueous suspension of ~3-12µm magnetic particles modified to provide surface primary amine groups. The non-spherical particles are irregular-shaped clusters of iron oxide with a broad size distribution. The irregular shape of these particles provides much greater surface area than similarly-sized spherical particles resulting in high binding capacities and efficient capture of target with conservative use of particles. The amine groups allow for the covalent attachment of ligand with retention of biological activity. Ligand can be covalently attached to BioMag® Maxi Amine by any of the reagents used to prepare affinity supports where the solid phase terminates with a primary amine group.[1] A suggested glutaraldehyde procedure is given below which follows the method of Weston and Avrameas.[2]

CHARACTERISTICS

Mean Diameter:	3-12µm
Particle Concentration:	~50 mg/mL

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.

Preparation of Solutions

Solution	Composition	Materials	Preparation Instructions
Coupling Buffer	0.01M pyridine	0.8mL pyridine	Add 0.8mL pyridine to 900mL distilled water. Adjust to pH 6.0 with 6N HCl. Fill to 1L with water.
Glutaral-dehyde	5% glutaral-dehyde	5mL 25% glutaraldehyde	In a hood, add 5mL of glutar-aldehyde to 20mL Coupling Buffer.
Glycine Quenching Solution	1M glycine	7.5g glycine	Dissolve 7.5g glycine in 90mL distilled water and adjust to pH 8.0 with 10N NaOH. Fill to 100mL with water.
Wash Buffer	0.01M Tris 0.1% NaN_3 0.1% w/v BSA 0.15M NaCl 0.001M EDTA	1.21g Tris 1g NaN_3 1g BSA 8.7g NaCl 0.37g EDTA	Dissolve solids in 900mL distilled water. Adjust to pH 7.4 with 10N NaOH or 6N HCl as required. Fill to 1L with water.

Activation

1. Transfer 10mL of BioMag® Maxi Amine (500mg particles) to a reaction flask that will easily contain the maximum volume of 50mL used in the coupling procedure. *Note:* A 50mL tissue culture flask or conical tube is typically used.
2. Add Coupling Buffer to a final volume of 50mL and shake vigorously. Then, using a magnetic flask separator, magnetically separate perpendicular to gravity until the supernatant is clear (approximately 10 minutes). Aspirate the supernatant, leaving the BioMag® as a wet cake on the container wall.
3. Repeat Step 2 three times.
4. Add 20mL of Glutaraldehyde (5% v/v final concentration) to the wet cake and shake vigorously.
5. Rotate at room temperature for 3 hours.
6. Magnetically separate perpendicular to gravity and aspirate the unreacted glutaraldehyde.
7. Repeat Step 2 four times.

Protein Coupling

1. Add 25-100mg of protein to 10mL of Coupling Buffer. *Note:* For monoclonal antibodies which may be expensive and are supplied at low concentrations (1mg/mL), a carrier protein, such as BSA, may be added to increase protein concentration. Various classes of monoclonal antibodies have been successfully coupled to BioMag® by offering 15mg of the monoclonal antibody with 100mg of BSA to 500mg of BioMag® Amine particles. The total volume of the suspension should be about 15mL. Shake vigorously or vortex.
2. Remove 75µL of the protein solution and add it to 1mL of Coupling Buffer. Label as Pre-Coupling Solution. Set aside for Coupling Efficiency Determination.
3. Add the remaining protein solution to the glutaraldehyde-activated BioMag® from Activation, Step 7. Shake vigorously and rotate 16-24 hours at room temperature.
4. Magnetically separate and save the supernatant. Label as Post-Coupling Solution and set aside for Coupling Efficiency Determination.
5. Add 50mL of Glycine Quenching Solution and shake vigorously. Rotate 30 minutes at room temperature.

Washing and Diluting Coupled Particles

1. Magnetically separate the particles and aspirate the supernatant.
2. Add 50mL of Wash Buffer and shake vigorously or carefully vortex.
3. Magnetically separate perpendicular to gravity, aspirate, and save the supernatant. Add to Post-Coupling Solution and set aside for Coupling Efficiency Determination.
4. Repeat Steps 2-3 three times.
5. Store the coupled BioMag® at 2-8°C as a suspension in Wash Buffer.

Coupling Efficiency Determination

1. Set spectrophotometer wavelength to 280nm. Blank with the Coupling Buffer.
2. Measure the absorbance of the Pre-Coupling Solution. A further dilution may be necessary to read an absorbance depending upon

the amount of protein added. Recall that the initial dilution made was 75µL in 1mL; a dilution factor (D) of 13.3.

3. Measure the absorbance of the Post-Coupling Solution. A dilution may be necessary to read the absorbance.
4. Calculate the coupling efficiency, expressed as the % Protein Uptake, as follows. Typical values of Protein Uptake are >60%.

$$\frac{[(\text{A280 Pre-Coupling Solution} \times D) - (\text{A280 Post-Coupling Solution} \times D)] \times 100}{(\text{A280 Pre-Coupling Solution} \times D)}$$

NOTES

1. Phosphate buffer (0.01M, pH 7) 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® 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 need 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® can make magnetic materials appear unstable in some applications.
3. Prolonged vigorous shaking or vortexing should be used to resuspend BioMag® after magnetic separation or settling with gravity.

REFERENCES

1. **Avrameas S**. 1969. Coupling of enzymes to proteins with glutaraldehyde. Use of conjugates for the detection of antigens and antibodies. *Immunochemistry*, 6(1): 43-52.
2. **Weston PD, Avrameas S**. 1971. Proteins coupled to polyacrylamide beads using glutaraldehyde. *Biochem Biophys Res Commun*, 45(6): 1574-1580.

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.

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

ORDERING INFORMATION

Cat. Code	Description	Sizes
BMM40	BioMag® Maxi Amine	10mL
AA012	Glutaraldehyde, EM Grade, 25%	10 x 10mL (ampoules)

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