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

**Description**

BioMag Maxi Carboxyl consists of an aqueous suspension of magnetic iron oxide particles modified to provide carboxyl groups. The carboxyl groups are sterically unencumbered, permitting the covalent attachment of proteins or ligands with retention of biological activity. Proteins or ligands can be covalently attached to BioMag Maxi Carboxyl by any of the reagents used to prepare affinity supports where the solid phase terminates with a carboxyl group.

**Characteristics**

Mean Diameter: 3-12µm  
 Concentration: 20mg/mL

**Procedure**

Researchers are advised to optimize the use of particles in any application.

**Preparation of Solutions**

| <u>Solution</u> | <u>Composition</u>   | <u>Materials</u>   | <u>Comments</u>  |
|-----------------|--|--|--|
| Coupling Buffer | 0.01M K <sub>2</sub> HPO <sub>4</sub><br>0.15M NaCl                              | 1.74g K <sub>2</sub> HPO <sub>4</sub><br>8.7g NaCl                     | Add solids to H <sub>2</sub> O. Adjust to pH 5.5.<br>Adjust to 1L.                   |
| Coupling Agent  | EDAC   | 40mg/70mL H <sub>2</sub> O   | Store at -20°C, dessicated, when not in use.<br>Warm to room temperature before use. |
| Wash Buffer     | 0.01M Tris<br>0.15M NaCl<br>0.1% w/v BSA<br>0.1% NaN <sub>3</sub><br>0.001M EDTA | 1.21g Tris<br>8.7g NaCl<br>1g BSA<br>1g NaN <sub>3</sub><br>0.37g EDTA | Dissolve solids. Adjust to pH 7.4 with NaOH<br>or HCl. Adjust to 1L with water.      |

**Activation**

1. Transfer 10mL of BioMag Maxi Carboxyl (200mg particles) to a reaction flask which will easily contain the maximum volume of 20mL used in the Protein Coupling procedure.
2. Add Coupling Buffer to a final volume of 20mL. Shake vigorously and magnetically separate, placing the flat side of the vessel alongside the magnetic separator, until 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. Suspend particles in 10mL of Coupling Buffer.

**Protein Coupling**

1. Add 4mL of Coupling Agent to particles and stir briefly.
2. Add 10mg of protein dissolved in no more than 10mL of water.
3. Stir and maintain the pH between 4.5-6.0 with 0.1M HCl for 30-60 minutes.

**Washing and Diluting Coupled Particles**

1. Magnetically separate and aspirate the supernatant.
2. Add approximately 20mL of Wash Buffer and shake vigorously or vortex.
3. Repeat Steps 1-2, three times.
4. Store the coupled particles as a suspension in Wash Buffer at 4°C.

**Testing for Binding Activity**

The coupled BioMag can now be assayed for the desired biological activity. For example, if an antibody has been coupled, the binding of a labeled antigen can be ascertained. *Note:* BioMag may have to be diluted before use.

**Notes**

1. Avoid 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). Amine or carboxyl groups containing buffers can be used as Wash Buffers. Phosphate is also satisfactory for use in the Wash Buffer in place of Tris.
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 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.

**Storage and Stability**

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

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

**Ordering Information**

| <b>Catalog Code</b> | <b>Description</b>    | <b>Size</b> |
|---------------------|-----------------------|-------------|
| BMM30               | BioMag® Maxi Carboxyl | 10mL        |

Order online anytime at [www.bangslabs.com](http://www.bangslabs.com).