Product Data Sheet 570

BioMag® Carboxyl

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B E A D S ● A B O V E T H E R E S T ™

DESCRIPTION

BioMag® 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® Carboxyl by any of the reagents used to prepare affinity supports where the solid phase terminates with a carboxyl group.

CHARACTERISTICS

Mean Diameter:	~1.5µm
Particle Concentration:	20 mg/mL
Surface Titration:	~240 µmol/g, ~4.8µmol/mL

MATERIAL

Material Supplied

- BioMag® Carboxyl: 10mL of aqueous suspension (pH 7.0) and ~200mg of BioMag®

Material Required

- Reaction flask
- BioMag® magnetic separator
- Sodium azide (NaN_3)
- Sodium chloride (NaCl)
- 1-ethyl-3-(3-dimethyaminopropyl) carbodiimide (EDAC)
- Tris base
- Bovine Serum Albumin (BSA)
- Potassium phosphate dibasic (K_2HPO_4)

PROCEDURE

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

Preparation of Solutions

Solution	*Composition*	*Materials*	*Preparation Instructions*
Coupling Buffer	0.01M K_2HPO_4 0.15M NaCl	1.74 K_2HPO_4 8.7g NaCl	Add solids to H_2O. Adjust to pH 5.5. Adjust to 1L. 900mL distilled water. Adjust to pH 6.0 with 6N HCl. Fill to 1L with water.
Coupling Agent	EDAC	40mg/70mL H_2O	Unstable; make just prior to use.
Wash Buffer	0.01M Tris 0.15M NaCl 0.1% w/v BSA 0.1% NaN_3 0.001M EDTA	1.21g Tris 8.7g NaCl 1g BSA 1.0g NaN_3 0.37g EDTA	Dissolve solids. Adjust to pH 7.4 with NaOH or HCl as required. Adjust to 1L with water.

Activation

1. Transfer 10mL of BioMag® Carboxyl 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. Aspirate the supernatant, leaving the BioMag® as a wet cake on the container wall.
3. Repeat Step 2, three times.
4. Suspend BioMag® in 10mL of Coupling Buffer.

Protein Coupling

1. Add 4mL of Coupling Agent to BioMag® and stir briefly.
2. Add 10mg of protein dissolved in no more than 10mL of water.
3. Stir and maintain 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.
5. Store the coupled BioMag® at 2-8˚C as a suspension in Wash Buffer.

Testing for Binding Activity

The coupled BioMag® can now be assayed for the desired biological activity. For example, if antibody has been coupled, the binding of a labeled antigen can be ascertained. 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 steps. 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.
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 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 BioMag® may result in irreversible aggregation and loss of binding activity. Centrifugation may be used only if it is the last step in a procedure such that resuspension of BioMag® is not required.

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	Sizes
BM570	BioMag® Carboxyl	10mL or 100mL

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