

TECHNICAL DATA SHEET 570

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BioMag[®] Carboxyl

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. A typical carbodiimide coupling procedure is given below. BioMag Carboxyl can also be used for DNA purifications.

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) with ~200mg of BioMag

Material Required

- Reaction flask
- BioMag magnetic separator (Cat. #84101S): permanent magnet to remove BioMag from suspension
- Sodium azide (NaN₃)
- Sodium chloride (NaCl)
- 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC)
- Tris base
- Bovine Serum Albumin (BSA)
- Potassium phosphate dibasic (K₂HPO₄)

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>Preparation Instructions</u>
Coupling Buffer	0.01M K ₂ HPO ₄ 0.15M NaCl	1.74 K ₂ HPO ₄ 8.7g NaCl	Add solids to H ₂ O. Adjust to pH 5.5. Adjust to 1L.
Coupling Agent	EDAC	40mg/70ml H ₂ O	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.

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Activation

1. Transfer 10ml of BioMag Carboxyl to a reaction flask which will easily contain the maximum volume of 20ml used below.
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 4°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 4°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.

Safety

This particle suspension contains sodium azide. 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.

Ordering Information

Catalog Number	Description	Sizes
84125	BioMag® Carboxyl	10ml or 100ml

To Order

In The U.S. Call: 1-800-523-2575 • 215-343-6484
In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767
In Germany FAX: (49) 6221-764620

Order online anytime at www.polysciences.com.