

TECHNICAL DATA SHEET 570

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BioMag® Carboxyl

Description

BioMag Carboxyl consists of an aqueous suspension of magnetic iron oxide particles coated to provide carboxyl groups. The carboxyl groups are sterically unencumbered, permitting the covalent attachment of proteins or ligands with retention of biological activity. Proteins and nucleic acids 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: ~1.6µm
Concentration: ~20mg/ml
Stoichiometry: ~240 µmol/g

Material Required/Supplied

Materials Supplied

BioMag Carboxyl: 10ml of aqueous suspension (pH 7) with approximately 200mg BioMag

Materials Required

Reaction Flask: a single, flat tissue culture vessel
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)

Solutions Needed

| Solution | Composition | Materials | Comments |
|-----------------|--|--|--|
| Coupling Buffer | 0.01M K ₂ HPO ₄ 0.15M NaCl | 1.74g 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 1g NaN ₃ 0.37g EDTA | Dissolve solids. Adjust to pH 7.4 with NaOH or HCl. Adjust to 1L with water. |

Procedure

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

Wash BioMag Before Use

1. Transfer 10ml of BioMag to a reaction flask which comfortably holds the maximum volume of 20ml used in the coupling procedure.
2. Add Coupling Buffer to a volume of about 20ml, shake vigorously, and place the flat side of the vessel alongside the BioMag Separator.
3. Aspirate the contents, leaving the BioMag as a wet cake on the container wall.
4. Repeat the washing procedure with three more additions of Coupling Buffer. Suspend BioMag in 10ml of Coupling Buffer.

Reaction of BioMag and Protein

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

Removal of Unreacted Protein

1. Magnetically separate the BioMag and aspirate the unreacted protein.
2. Add approximately 20ml of Wash Buffer and shake.
3. Magnetically separate, aspirate, and add Wash Buffer a total of four times.
4. Shake BioMag vigorously to disperse. Store reacted BioMag as a suspension in Wash Buffer at 4°C.

Testing for Magnetic Binding Activity

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

Notes

A. 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 group containing buffers can be used as Wash Buffers.

B. 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.

C. Prolonged, vigorous shaking should be used to break up BioMag after magnetic separation or settling with gravity.

Storage and Stability

Store at 4°C. Freezing, drying, or centrifuging of BioMag 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

| Cat. # | Description | Sizes |
|---------------|--------------------|--------------|
| 84125 | BioMag® Carboxyl | 10ml, 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

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