

Product Information

Amine-Terminated Magnetic Particles

Product Number **I 7643**

Product Description

Amine-Terminated Magnetic Particles are supplied as an aqueous suspension of magnetic iron oxide particles coated to provide primary amino groups. Proteins can be covalently attached to the particles by reagents commonly used to prepare affinity supports having a solid phase terminating with a primary amine.¹

The product is supplied as a suspension of primary amine coated superparamagnetic iron oxide particles, approximately 1 μm in size, at a concentration of 50 mg per ml in 1 mM EDTA, pH 7.0.

Extent of Activation: Approximately 12 μmoles per ml and 240 μm per gram.

Coupling Capacity: Typically 10 mg of protein per ml.

Precautions and Disclaimer

Solutions containing glutaraldehyde or pyridine are volatile and noxious. Perform operations with these solutions in a chemical fume hood.

Preparation Instructions

1. Coupling Buffer (0.01 M pyridine) – Add 0.8 ml of pyridine to 900 ml of distilled water. Adjust the pH to 6 with 6 N HCl. Add distilled water to a final volume of 1 liter.
2. 5% Glutaraldehyde Solution – In a hood, add 5 ml of 25% aqueous glutaraldehyde solution to 20 ml of Coupling Buffer.
3. Glycine Quenching Solution (1.0 M) – Dissolve 7.5 g of glycine in 90 ml of distilled water. Adjust the pH to 8 with 10 N NaOH. Add distilled water to a final volume of 100 ml.
4. Wash Buffer (0.01 M Tris Base containing 0.15 M NaCl, 0.1% (w/v) bovine serum albumin (BSA), 0.001 M EDTA, sodium salt and 0.1% (w/v) sodium azide) – Dissolve 1.21 g of Tris Base, 8.7 g of NaCl, 1 g of BSA, 0.37 g of EDTA, sodium salt and 1.0 g of sodium azide in 900 ml of distilled water. Adjust the pH to 7.4 with 10 N HCl. Add distilled water to a final volume of 1 liter.

Storage/Stability

Storage at 2 to 8 °C is recommended. Do not freeze.

Procedure

The following procedure is recommended for use with the coated particles. It is a glutaraldehyde procedure, which is a modification of the method of Weston and Avrameas.²

1. Particle Activation
 - a. Transfer 10 ml of the Amine-Terminated Magnetic Particle suspension to a reaction vessel (A 50 ml tissue culture flask or conical tube is typically used.).
 - b. Add Coupling Buffer to a final volume of 50 ml, shake vigorously to suspend particles, and then magnetically separate until the supernatant is clear (approximately 10 minutes). Aspirate the supernatant, leaving the particles as a wet cake on the container wall. Repeat three times.
 - c. Add 20 ml of the 5% Glutaraldehyde Solution to the wet cake, shake vigorously to resuspend the particles, and then gently shake at room temperature for three hours.
 - d. Magnetically separate and aspirate the supernatant containing the unreacted glutaraldehyde.
 - e. Wash particles as described in step 1.b.
2. Coupling of Protein
 - a. Dissolve 25 to 100 mg of protein in 10 ml of the Coupling Buffer. For monoclonal antibodies, which may be expensive and are supplied at low concentrations (1 mg/ml), a carrier protein such as BSA may be added to increase the protein concentration. Mix 15 mg of the monoclonal antibody with 100 mg of BSA and 500 mg of the particles. The total volume of the suspension should be about 15 ml.
 - b. Remove 75 μl of the protein solution and add Coupling Buffer (approximately 1 ml) until the A_{280} is in the range of 0.5 to 0.7. Label as "Pre-Coupling Solution". Set aside for later use in coupling efficiency determination.

- c. Add the remaining protein solution to the glutaraldehyde-activated magnetic particles from step 1.e. Shake vigorously to resuspend particles and then follow with gentle shaking for 16 to 24 hours at room temperature.
 - d. Magnetically separate the particles from the supernatant. Aspirate and save the supernatant. Label as "Post-Coupling Supernatant" and set aside for later use in the coupling efficiency determination.
 - e. Add 50 ml of the Glycine Quenching Solution and shake vigorously to resuspend the particles. Then shake gently for 30 minutes at room temperature.
3. Washing and Diluting Coupled Particles
 - a. Magnetically separate particles and aspirate supernatant.
 - b. Wash the particles as described in step 1.b.
 - c. Store the coupled particles at 4 °C as a suspension in the Wash Buffer or in a buffer compatible with the attached protein (pH should be in the range of 4 to 10). The coupled particles will settle with prolonged storage and should be shaken vigorously before use. Do not freeze or dry.
 4. Coupling Efficiency Determination
 - a. Set the spectrophotometer wavelength to 280 nm. Use the Coupling Buffer as a blank.
 - b. Measure the absorbance of the "Pre-Coupling Solution" from step 2.b. A dilution may be necessary to read an absorbance.
 - c. Measure the absorbance of the "Post-Coupling supernatant" from step 2.d. A dilution may be necessary to read the absorbance.
 - d. Calculate the coupling efficiency (% Protein Uptake).

% Protein Uptake =

$$\frac{(A_{280} \text{ Pre-Coupling Solution} \times D) - (A_{280} \text{ Post-Coupling Supernatant} \times D) \times 100}{(A_{280} \text{ Pre-Coupling Solution} \times D)}$$

D = dilution factor

% Protein Uptake should be greater than 60

- e. After the coupling efficiency has been determined, the particles may be used in the desired application.

Notes:

1. Phosphate buffer (0.01 M, pH 7.0) can be used as a Coupling Buffer, but with reduced coupling efficiency compared to pyridine buffer. Do not use primary amines, ammonium ions, or other strong nucleophiles in the Coupling Buffer. All coupling buffers should be used at minimal ionic strengths.
2. Buffers containing amines (Tris) or phosphate buffers can be used as wash buffers. Ionic strength has little or no effect once the protein is attached to the particles.
3. Some noncovalent adsorption invariably accompanies covalent coupling to the particles. Noncovalent adsorption may be reduced 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 include high salt concentration (1 M NaCl), mildly acidic or basic media, mildly elevated temperatures, or increased exposure time to the Wash Buffer. Dissociation of active, noncovalently adsorbed proteins can make magnetic the material appear unstable in some applications.
4. Prolonged, vigorous shaking should be used to resuspend particles after magnetic separation or settling with gravity.

References

1. Hermentin, P. et al., *Bioconjugate Chem.*, **1**, 411 (1990).
2. Weston, P. D., and Avrameas, S., *Biochem. Biophys. Res. Comm.*, **45**, 1574 (1971).

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