Steinbrenner Laborsysteme GmbH

magtivio

Technical Note

Covalent Coupling of Target Protein (Peptide) via C terminus on MagSi-S NH2 beads by Carbodiimide Method

I. General considerations

- For an optimal binding capacity of the molecule of interest to an amine activated surface use washing & binding buffer with the pH between pH 5.5 – 6.5.
- All buffers used for washing, activation or coupling may not contain carboxyl-groups, proteins or high salt conditions.
- In case of antibodies or proteins, we would recommend to use a minimum amount of 10 µg target protein per 1mg MagSi-S NH2 beads.
- Prepare the carbodiimide (EDC) solution fresh just before use.

II. Equipment and reagents

- MagSi-S NH2 1.0 (magtivio BV/ MD03005)

Wash & Coupling Buffer

- 0.1 M 2-(N-Morpholino)ethanesulfonic acid (MES), pH 6.0 (Sigma/ 69889-10G)

Water Soluble Carbodiimide

- N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)(VWR**/ 8510070025)**

Blocking & Storage Buffer

- 0.01 M PBS (Sigma/ P3813); 0.1 % gelatin from cold water fish skin (**Sigma/ G7765);** 0.05 % sodium azide (NaN3)(VWR/1066880100)

Wash buffer

- Phosphate buffered saline with Tween 20 (PBS/Tween)(Sigma/ P3563)
- Magnetic separator (e.g. Magnetic separator M12+12/ magtivio BV)
- Plastic Pasteur pipettes (VWR/ 6121684)
- Plastic centrifuge tubes, 2ml

- Eppendorf pipette
- Tube Rotator (e.g. VWR)
- Mini Vortex (e.g. VWR)

III. Work Protocol

The following protocol describes the coupling of biomolecules on 10 mg MagSi-S NH2 beads (1ml/10mg ml). This work protocol can be scaled up or down upon request.

1. Vortex the beads as in the closed bottle for 2min before use.
2. Transfer 1ml using an Eppendorf pipette
3. Rinse the MagSi-S NH2 beads with 2 ml 0.1 M MES buffer. Using the magnetic separator collect the beads. (Make sure that the bead collection time is long enough in order to enable entire beads collection/ until supernatant is clear) and then carefully remove the supernatant. Repeat 2x.
4. After the third rinse step re-suspend the beads in 0.25 ml MES buffer containing 10 mg EDC (stock solution EDC: 40 mg/ml). Add only freshly prepared EDC solution to the beads and vortex it for 20 s.
5. Add carboxyl group containing ligands (e.g. 40 µg protein/mg bead dissolved in ddH2O; 80µl from a stock solution of 5mg/ml) to the particles and vortex the bead suspension for 10 s. Complete the reaction end volume to 0.5ml with 0.1M MES. Mix the beads suspension on a rotator for 1 hour at room temperature.
6. Rinse the beads with 1 ml 0.01 M PBS containing 0.1 % gelatin from cold water fish skin. Using the magnetic separator collect the beads. (Make sure that the bead collection time is long enough in order to enable entire beads collection/ until supernatant get clear) and then carefully remove the supernatant. Repeat 2x.
7. Rinse the beads with 1 ml 0.01 M PBS containing 0.05% Tween 20. Repeat 2x.
8. Re-suspend and store the beads in 1 ml 0.01 M PBS, 0.1 % gelatin; 0.05 % sodium azide.

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