Steinbrenner Laborsysteme GmbH

magtivio

Technical Note

MagSi-S Epoxy - Product preparation and coupling guidelines

I. Introduction

This technical note is intended as a guideline for using MagSi-S Epoxy. The magnetic beads are supplied in organic solvent mix Dimethyl sulphoxide (DMSO): Tetrahydrofuran (THF) 1:1, v/v to ensure stability of the epoxy-activated surface. This note describes the preparation of the product and how to covalently bind target biomolecules.

II. Considerations before coupling

- In this protocol different options are given for coupling and blocking buffer. The purpose of providing different options, is to develop the best suitable protocol for the target biomolecule of interest.

- The protocol can be scaled up and down, depending on the initial volume of magnetic beads. For different volumes of magnetic beads (wheter or not in microcentrifuge format) have different separation times. Always make sure your supernatant is clear before aspirating.

- **biomolecule amount**

  The amount of biomolecules which will be coupled to the MagSi-S Epoxy beads is depending on their size and the initial amount of beads. Carefully consider the needed amount of target biomolecules and the amount of magnetic beads. For example for small peptides it is possible to couple 100 µg/mg beads, larger proteins (for example: antibodies) can be coupled in lowwer amounts 10-50 µg/mg beads.

- **blocking options**

  This protocol describes 2 different blocking options. It is recommended initially try out option A. In case of success, in next experiments it is possible to explore option B as this is a cost and time saving alternative.

III. Equipment and consumables

- **Magnetic separator**
  - MM-Separator M12+12 (magtivio B.V. Art.No. MD90001): Magnetic separator for use of MagSi beads in 12x 1.5 mL and 12x 2 mL tubes, made of acryl (non alcohol resistant).
  - MM-Separator M12+12 P (magtivio B.V., Art.No. MDMG0001): Magnetic separator for use of MagSi beads in 12x 1.5 mL and 12x 2 mL tubes, made of POM (alcohol resistant).
- Vortexer
- Tube rotator
- Micropipettes (variable volumes)
- Microcentrifuge tubes (1.5 mL, 2 mL)

IV. Required materials

- MagSi-S Epoxy (10 mg/mL), supplied in DMSO:THF 1:1, v/v
- Methanol, technical grade
- Demineralized water
- PBS
- sodium bicarbonate
- ethanolamine
- NaCl
- Tween 20
- Tris Hcl
- Glycine
- casein

V. Solutions to be prepared by the user

- Preparation Buffer: 0.01M PBS, pH 7.4
- Coupling Buffer: 0.01M PBS, pH 7.4 containing 1M NaCl or 0.1M PBS, pH 7.4-8.5 or 0.1M sodium bicarbonate buffer pH 8.0-9.5
- Wash & Blocking Buffer: 1 wt/v% Ethanolamine in 0.01 M PBS with or without 1M NaCl/ 0.05 wt/v % Tween 20 or 10mM TRIS, pH 8.5 containing 0.05 wt/v% Tween 20 or 1 wt/v% Glycine in 0.01 M PBS, pH 7.4 with and/or without 1M NaCl/ 0.05 wt/v % Tween 20
- Storage Buffer: 0.01M PBS, 0.05 wt/v% NaN3, 0.1 wt/v% Glycine; (optional 0.1 wt/v% BSA and/or gelatine and/or casein)

VI. Preparation procedure

1. Resuspend the beads by mixing intensively for at least 2 minutes before use.
2. Transfer the desired volume of **MagSi-S Epoxy** for coupling to a micro-centrifuge tube.
3. Place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the storing solution by aspirating slowly the supernatant.
4. Remove the tube from the magnetic separator and add **Methanol** using a volume 3 times the volume of the initial beads suspension taken in step 2. Vortex shortly to resuspend the beads.
5. Place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the Methanol solution by aspirating slowly the supernatant. Repeat steps 4 and 5, two times (3 times in total).
6. Remove the tube from the magnetic separator and add **Preparation Buffer** using a volume 3 times the volume of the initial beads suspension taken in step 2. Vortex shortly to resuspend the beads.
7. Place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the Preparation buffer by slowly aspirating the supernatant.
8. Repeat steps 6-7; 4 times (5 times in total).

VII. Coupling procedure

9. Remove the tube from the magnetic separator and add **Coupling Buffer**, using a volume 3 times the volume of the initial beads suspension taken in step 2. Vortex shortly to resuspend the beads.
10. Place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the coupling buffer by slowly aspirating the supernatant.
11. Repeat step 9-10; 2 times (3 times in total).
12. Finally, resuspend the beads in **Coupling Buffer** in a volume that the beads end up in a concentration of 20-50mg/ml. The product is now ready for coupling of the target biomolecule.
13. Add the target biomolecule for covalent coupling dissolved in demineralized water or in an aqueous buffer that contains no NH2 groups and has a pH between 7 and 8. The amount of target molecule is depending on the application (e.g. ~60-150 µg peptides or proteins per mg beads, ~10-30 µg antibody per mg beads).
14. Incubate with gentle mixing for 8-16h at RT, or at 2-8°C for sensitive target biomolecules. Increasing the incubation time will increase the covalent coupling yield.

    Note: Choose option A or B for immobilization. See chapter VIII: Blocking procedure and storage.
15. After incubation, place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the solution by slowly aspirating the supernatant.

16. Remove the tube from the magnetic separator and add **Wash & Blocking Buffer** using a volume 3 times the volume of the initial beads suspension taken in step 2. Vortex shortly to resuspend the beads.

17. Repeat step 15-16; 2 times (3 times in total).

18. Place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the solution by slowly aspirating the supernatant.

VIII. Blocking procedure and storage

Option A - Blocking following the target immobilization / step 18

19. Add **Wash & Blocking Buffer**, using the same volume of the initial beads suspension taken in step 2. Incubate for 4h at RT on a tube rotator.

20. Place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the Wash & Blocking buffer by slowly aspirating the supernatant.

21. Remove the tube from the magnetic separator and add **Storage Buffer**, using the same volume of the initial beads suspension taken in step 2. Vortex shortly to resuspend the beads.

22. Repeat step 20-21; 2 times (3 times in total).Place the tube in the magnetic separatorand wait until a clear supernatant is obtained (ca. 3 min). Discard the Wash & Blocking buffer by slowly aspirating the supernatant.

23. Finally store the beads in **Storage Buffer** (Optionally containing 0.1 wt% BSA and/or 0.1 wt% gelatine and/or 0.1 wt% casein).

Option B – Blocking on top of the target immobilization / step 14

24. After incubation, add on top of the bead suspension sample the **Wash & Blocking buffers** at a volume ratio 1:10 to 1:5 and incubate at RT for another 4h.

25. Place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the **Wash & Blocking buffer** by slowly aspirating the supernatant.

26. Remove the tube from the magnetic separator and add **Storage Buffer**, using the same volume of the initial beads suspension taken in step 2. Vortex shortly to resuspend the beads

27. Repeat step 25-26; 2 times (3 times in total).Place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the **Wash & Blocking buffer** by slowly aspirating the supernatant.

28. Finally store the beads in **Storage Buffer** (Optionally containing 0.1 wt% BSA and/or 0.1 wt% gelatine and/or 0.1 wt% casein).