Technical Note

MagSi-S Tosyl - Product preparation and coupling guidelines

I. Introduction:

This technical note is intended as a guideline for using **MagSi-S Tosyl**. The product is supplied in an organic solvent mix of dimethyl sulphoxide and tetrahydrofuran to ensure stability of the tosyl-activated surface. This note describes the preparation of the product and how to covalently bind 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 from 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 from POM (alcohol resistant).
- Vortexer
- Micropipettes (variable volumes)
- Microcentrifuge tubes (1.5 mL, 2 mL)
- Tube rotator

IV. Required materials

- MagSi-S Tosyl (10 mg/mL), supplied in DMSO:THF 1:1, v/v
- Methanol, technical grade
- PBS
- $(NH_4)_2SO_4$
- Borate buffer
- TRIS
- Glycine
- Ethanolamine
- Tween 20
- BSA

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 3M $(NH_4)_2SO_4$ **or** 0.1M Borate buffer, pH 9.3
- **Blocking Buffer**: 0.2M TRIS, pH 8.5 containing 0.1% (wt/v) BSA **or** 0.01M PBS with 1% (wt/v) glycine or ethanolamine
- **Storage Buffer**: 0.01M PBS, pH 7.4 containing 0.05 % (wt/v) Tween 20 (optional:0.01% (wt/v) BSA and/or 1% (wt/v) glycine)

VI. Preparation procedure:

1. Resuspend the beads by vortexing intensively for at least 2 minutes before use.
2. Transfer the desired volume of MagSi-S Tosyl 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 slowly aspirating the supernatant.
4. Remove the tube from the magnetic separator and add three volumes of methanol for every volume of bead suspension initially taken in step 2. Vortex intensively for at least 30 seconds.
5. Place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the methanol solution by slowly aspirating the supernatant. Repeat steps 4 and 5 two times (three times in total).

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6. Remove the tube from the magnetic separator and add three volumes of Preparation Buffer for every volume of bead suspension initially taken in step 2. Vortex intensively for at least 30 seconds.

7. Place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the Preparation Buffer solution by slowly aspirating the supernatant. Repeat steps 6 and 7 two times (three times in total).

VII. Coupling procedure:

8. Remove the tube from the magnetic separator and add three volumes of **Coupling Buffer** for every volume of bead suspension initially taken in step 2. Vortex intensively for at least 30 seconds.

9. Place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the **Coupling Buffer** solution by slowly aspirating the supernatant. Repeat steps 8 and 9 two times (three times in total).

10. Finally, add an appropriate volume of **Coupling Buffer** and resuspend the beads by vortexing in order to obtain a recommended final bead concentration of 20-50 mg/mL.

11. Add the target biomolecule for covalent coupling. The amount of biomolecule depends on the application (approximately 80-120 µg peptides/proteins or 10-30 µg antibody per mg beads).

12. Incubate with gentle mixing for 16-36h at RT, or at 2-8°C for sensitive biomolecules. Increasing the incubation time will increase the covalent coupling yield.

    Note: choose option A or B for immobilization. See chapter VII. Blocking procedures and storage.

13. After incubation, place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the **Coupling Buffer** solution by slowly aspirating the supernatant.

14. Remove the tube from the magnetic separator and add three volumes of **Coupling Buffer** for every volume of bead suspension initially taken in step 2. Vortex intensively for at least 30 seconds. Repeat steps 13 and 14 two times (three times in total).

VIII. Blocking procedures and storage

Option A - Blocking following the target immobilization / step 14

15. Add one volume of **Blocking Buffer** for every volume of bead suspension initially taken in step 2 and resuspend by vortexing intensively for at least 30 seconds. Incubate for 24h at RT with gentle mixing on the rotator.

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

17. Remove the tube from the magnetic separator and add three volumes of **Storage Buffer** for every volume of bead suspension initially taken in step 2. Vortex intensively for at least 30 seconds. Repeat steps 16 and 17 two times (three times in total).

18. Finally store the beads in **Storage Buffer.**

Option B - Blocking on top of the target immobilization / step 12

19. After the incubation in step 12, add 1/10 to 1/5 volume of **Blocking Buffer** for every volume of bead suspension initially taken in step 2. Continue incubation under gentle rotation for another 4 hours.

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

21. Remove the tube from the magnetic separator and add three volumes of **Storage Buffer** for every volume of bead suspension initially taken in step 2. Vortex intensively for at least 30 seconds. Repeat steps 20 and 21 three times (four times in total).

22. Finally store the beads in **Storage Buffer**.