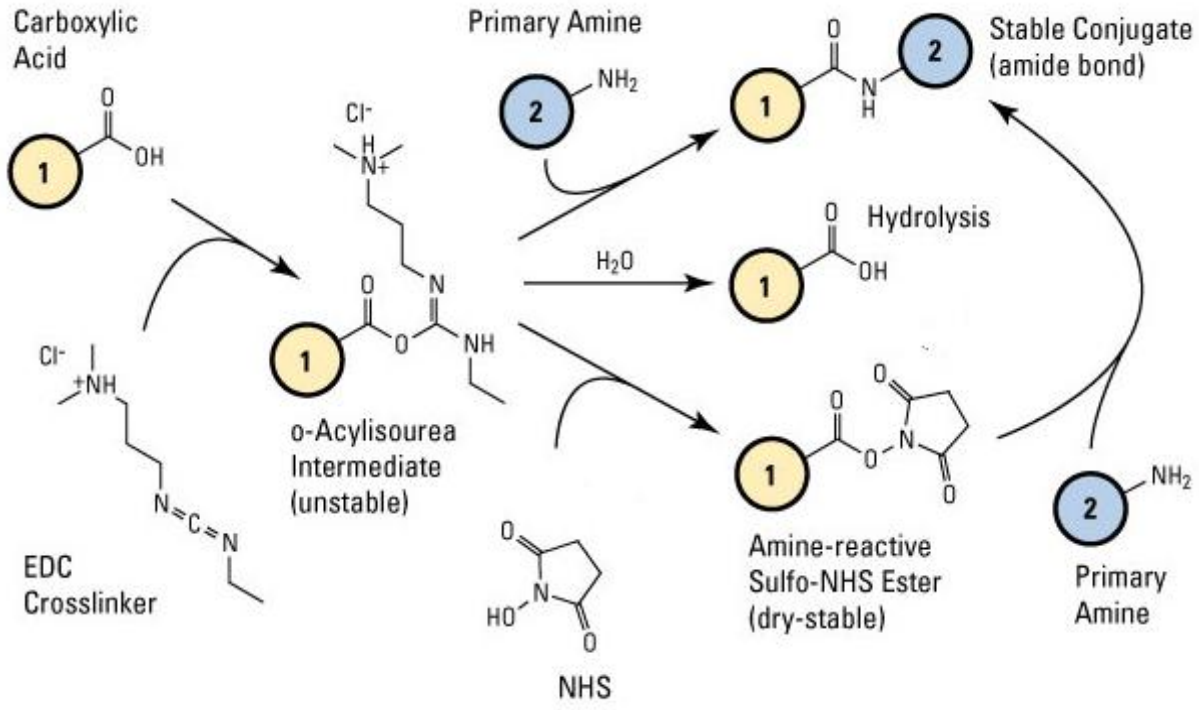
Technical Note

Covalent Coupling on MagSi-S COOH terminated beads by Carbodiimide Method

I. Introduction

This protocol describes the covalent coupling of amino-group containing biomolecule ligands such as antibodies, proteins or low molecular mass target molecule ligands to MagSi-S COOH terminated beads using carbodiimide method.

Carbodiimides react with the terminal carboxyl groups from the magnetic beads **(1)** to form highly reactive O-acylisourea derivatives that further rapidly react with amino groups of the biomolecule and/or low molecular mass target molecules ligands **(2)**, as presented in the below figure.



It is recommend to couple biomolecules via the 2-step method in order to prevent crosslinking effects.

II. Considerations before coupling

- All buffers used for activation or coupling purposes **must not contain molecules with amino groups** (like e.g. demineralized water or 0.01M PBS, pH 7.4), proteins additives or high ionic strength compositions, since they interfere with the overall coupling efficiency.

- For antibodies / proteins, it is recommended to use a minimum amount of 10-100 µg antibody/protein per 1 mg MagSi-S COOH beads. In general, the amount of protein which is immobilized to the beads should be optimized by the customer according to the proposed targets. The higher the amount of antibody/protein per mg of beads, the higher the degree of surface coating with the protein and implicitly the higher the activity is obtained but in the same time some steric hindrance effects might be faced.

- Prepare the NHS/EDC solutions immediately before use and mix the volume rapidly into the reaction tube.

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

- Micropipettes (variable volumes)

- Microcentrifuge tubes (1.5 mL, 2 mL)

- Tube rotator

IV. Required materials

- MagSi-S COOH (10mg/ml), supplied in PBS
- 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)
- N-hydroxysuccinimide (NHS)
- 2-(N-Morpholino)ethanesulfonic acid (MES)
- PBS
- TRIS
- Ethanolamine
- Tween 20
- BSA
- sodium azide

V. Solutions to be prepared by the user:

- Activation Buffer: 0.1M 2-(N-Morpholino)ethanesulfonic acid (MES), pH 5.5
- Wash Buffer 1: 0.01M 2-(N-Morpholino)ethanesulfonic acid (MES), pH 5.5
- Wash Buffer 2: 0.01M PBS, 0.05% (wt/v) Tween 20
- Coupling buffer: 0.01M Phosphate Buffered Saline (PBS), pH 7.04
- Blocking buffer: 0.1M Tris Hydroxymethylaminoethane (TRIS buffer), pH 8.0 or 1% (wt/v) Ethanolamine in 0.01M PBS, pH 7.4
- Storage Buffer: 0.01M PBS, 0.05% (wt/v) Tween 20, (0.1% BSA), 0.05% (wt/v) sodium azide

VI. Coupling procedure

The protocol describes the coupling of target biomolecules MagSi-S COOH (10 mg/ml) beads. It can be scaled up and/or down by adjusting volumes of required reagents.

1. Resuspend the beads by vortexing intensively for at least 2 minutes before use.
2. Transfer the desired volume of **MagSi-S COOH** 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 storage solution by slowly aspirating the supernatant.
4. Remove the tube from the magnetic separator and add 3 volumes of **Activation Buffer**, for every volume of bead suspension initially taken in step 2. Vortex intensively for at least 30 seconds.
5. Repeat steps 3 and 4 two times (three times in total).
6. It is recommended to perform coupling with a concentration of 20-50 mg beads/mL. Therefore resuspend the magnetic beads in ¼ volume **Activation Buffer** for the initial volume of bead suspension taken in step 2.
7. Prepare an NHS solution in **Activation Buffer** with a concentration of 20-40 mg NHS / mL activation buffer.
8. Prepare an EDC solution in **Activation Buffer** with a concentration of 10-40 mg EDC / mL activation buffer.
9. First add the NHS solution in **Activation Buffer** followed by EDC in **Activation Buffer** at an end concentration 10-40 mg beads/mL. The total volume for this activation step is the same volume as the initial bead solution taken in step 2.

   Note: The ratio between NHS and EDC can be optimized. It is recommended to start at a ratio of 1:1.
10. Place the tube with beads on a rotator for 15 minutes at room temperature (RT).
11. After incubation, place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min). Discard the buffers by slowly aspirating the supernatant.
12. Remove the tube from the magnetic separator and wash the beads 1 time with **Wash Buffer 1** with the initial volume taken in step 2. Vortex the sample shortly to resuspend the beads and place the tube in the magnetic separator and wait until a clear supernatant is obtained. Discard Wash Buffer 1 by slowly aspirating the supernatant. The beads contain now activated COOH groups that can bind proteins/amine containing target molecules.

13. Remove the tube from the magnetic separator and add to the activated beads, amine group containing ligands (e.g. 10-100 µg protein/mg bead). To adjust the final bead concentration, add **Coupling buffer** to a final coupling volume of half of the initial sample volume taken in step 2.

14. *) Place the tube with the beads on a rotator for 60 minutes at room temperature (RT).

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

16. Remove the tube from the magnetic separator and wash the beads, with a volume initially taken in step 2, with **Wash Buffer 2**. Vortex shortly to resuspend the beads.

17. Place the tube in the magnetic separator and wait until a clear supernatant is obtained (ca. 3 min.). Discard wash buffer 2 by slowly aspirating the supernatant.

18. Repeat step 16 and 17 two more times (3 times in total). Resuspend the particles in **Blocking buffer** (initial volume taken in step 2.)

19. Place the tube with the beads on a rotator for 2 hours at RT.

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

21. Remove the tube from the magnetic separator and add **Wash Buffer 2** (same initial volume as taken in step 2.), vortex shortly to resuspend the beads.

22. Repeat step 20 - 21 two more times (total of 3 washes).

23. Finally store the beads in **Storage Buffer** (initial volume taken in step 2).

**) The steps described under 14-18 are optional. Some applications require an additional blocking step after immobilization.*