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Technical Note

Coupling of oxidized glycoproteins (e.g. antibodies) onto MagSi-S Hydrazide

MagSi-S Hydrazide - Product preparation and coupling guidelines

I. Introduction

MagSi-S Hydrazide contains hydrazide groups on the bead surface that react with oxidized carbohydrates containing ether aldehyde or ketones to form stable, covalent bonds via hydrazone new group formation. Using this chemistry, immunoglobulins and glycoproteins can be immobilized onto the MagSi-S Hydrazide bead surface through oxidized protein carbohydrate side-chains. Oxidation of the carbohydrate side-chains with sodium periodate enables generation of carbonyl groups which can be later used for the oriented coupling of the target glycoproteins onto the MagSi-S Hydrazide beads. Such specific oriented coupling onto the bead surface of an antibody results in a maximum availability of antigen binding and therefore reduces the steric hindrance when compared to immobilizations that lead to a random distribution onto the bead surface.

Features & Benefits

- A convenient method to immobilize glycoproteins (e.g. antibodies) onto MagSi-S Hydrazide modified bead surface in a specific/oriented fashion.
- The proposed protocol is flexible and therefore offers possibilities for further optimization towards a specific application.

II. Materials needed

- MagSi-S Hydrazide (10 mg/mL)
- Oxidation Reagent: Sodium periodate ($NaIO_4$)
- Quenching Reagent: Polyethylene glycol 400 (PEG 400)
- Membrane filtration dialysis unit, 2 mL, 10 kDa cut-off, (e.g. Vivaspin spin filter-VS0201, Sartorius)
- Magnetic separator; (e.g. MM separator M12+12, magtivio B.V.)
- Rotator
- Vortexer
- Microcentrifuge tubes; 2 mL and 10 mL
- Micropipettes (variable volumes)

III. Buffers

- Wash & Coupling Buffer: 0.02 M PBS, pH 7.4
- Oxidation Buffer: 0.1 M Sodium acetate buffer, pH 5.5
- Blocking Buffer: 0.1 M D-glyceraldehyde in Wash & Coupling Buffer
- Storage Buffer: 0.01 M PBS containing 0.1% (wt/v) Glycine, 0.05% (wt/v) NaN_3, pH 7.4

IV. Oxidation of the glycoprotein (e.g. Antibody) prior coupling*

*The protocol refers to 5 mg antibody. The oxidation is light sensitive and must be conducted in a dark environment.

1. Prior to oxidation it is important that the original buffer of the antibody is exchanged to **Oxidation Buffer** (0.1 M Sodium acetate buffer, pH 5.5) using a membrane filtration dialysis unit. Finally resuspend the antibody in 1 mL **Oxidation Buffer**. The buffer exchange operation has to be conducted according to the manufacturer's instructions of the membrane filtration dialysis unit.

2. Add the antibody solution in Oxidation Buffer to a 10 mL centrifuge tube containing 5 mg **Oxidation Reagent** (approx. 20 mM). Close the tube and mix gently by inverting the tube 10 times to dissolve it. Wrap the sample tube with aluminium foil in order to protect the sample from light and place it on the rotator.

3. Incubate the sample at RT for 30-60 min under rotation.

4. Stop the reaction by adding 0.25 mL **Quenching Reagent** to the tube containing the oxidized antibody.

5. Place the tube on the rotator and incubate for 5 min at RT under rotation.

6. Remove the unreacted excess of **Oxidation Reagent** and **Quenching Reagent** by desalting and buffer exchange using a membrane filtration dialysis unit. Finally resuspend the oxidized antibody in 2 mL of **Wash & Coupling Buffer**. The final concentration of the oxidized antibody is now appr. 2.5 mg/mL.

V. Coupling procedure**

**The protocol refers to coupling appr. 0.5 mg antibody; the protocol is scalable.

7. Transfer 1 mL of **MagSi-S Hydrazide** (10 mg/mL) beads into a 2 mL microcentrifuge tube.

8. Collect the beads using the magnetic separator and discard the supernatant.

9. Rinse the beads by resuspending in 1 mL **Wash & Coupling Buffer**. Collect the beads again with the magnetic separator and discard the supernatant. Repeat two times for a total of 3 washes.

10. Resuspend the beads in 0.3 mL **Wash & Coupling Buffer**. Mix the beads with 0.2 mL oxidized antibody solution.

11. Incubate overnight or for at least 8 hours at RT under rotation.

12. After completing the coupling reaction, collect the beads using the magnetic separator and discard the supernatant.

13. Rinse the beads by resuspending in 1 mL **Wash & Coupling Buffer.** Collect the beads again with the magnetic separator and discard the supernatant. Repeat two times for a total of 3 washes.

14. Resuspend the decanted beads in 0.3 mL **Wash & Coupling Buffer** and add 0.2 mL of **Blocking Buffer**.

15. Incubate for 1h at RT under rotation.

16. After incubation, collect the beads using the magnetic separator and discard the supernatant.

17. Rinse the beads by resuspending in 1 mL **Storage Buffer.** Collect the beads again with the magnetic separator and discard the supernatant. Repeat two times for a total of 3 washes.

18. Finally store the beads in 1 mL Storage Buffer

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