Covalent Coupling Procedure on SiMAG-Hydrazide

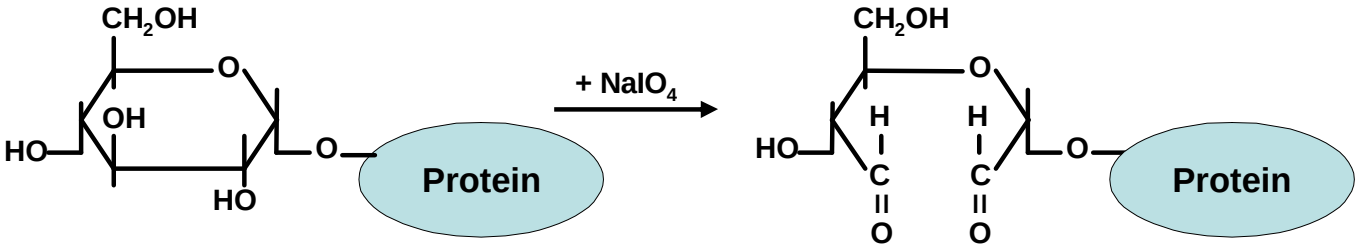
Introduction:

This procedure describes the covalent coupling of aldehyde or ketone group containing ligands by the formation of stable hydrazone linkages on **SiMAG-Hydrazide**.

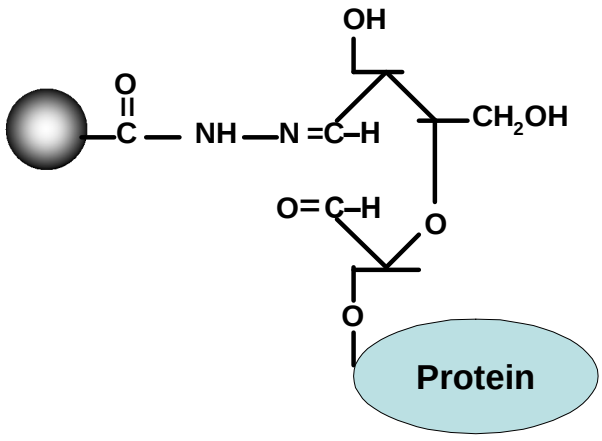
Glycoproteins can be immobilized by oxidation with sodium periodate to generate formyl groups on their carbohydrate chains.

This coupling method is a powerful way to immobilize proteins and leave critical active sites free.

Oxidation of carbohydrate residues



Forming of aldehyde groups



Site-directed immobilization through carbohydrates

Equipment and reagents:

- **SiMAG-Hydrazide**
- **Wash & Coupling Buffer:**

  0.1 M sodium phosphate buffer, pH 7.0

- **Oxidation Reagent:**

  sodium meta-periodate ($NaIO_4$)

- **Blocking Buffer:**

  0.1 M D-glyceraldehyde in **Wash & Coupling Buffer**

- **Storage Buffer:**

  PBS, 0.1 % BSA, 0.05 % sodium azide

- **Magnetic Separator** (**e.g. MagnetoPURE,** Product Number: MP-10)

Technical Note:

- The reaction is light sensitive and should be performed in the dark.
- Coupling efficiencies hydrazide modified magnetic particles depend on the structure and the size of the target glycoprotein. The user should empirically optimize the concentration of the protein. We recommend starting with ~1 mg oxidized protein for 10 mg **SiMAG-Hydrazide**.

The following protocol describes the coupling of biomolecules on *10 mg* particles. The procedure can be scaled up by adjusting volumes of required reagents.

Protocol:

Oxidation:

1. Dissolve 5 -10 mg glycoprotein in 1 ml 0.1 M Wash & Coupling Buffer.

2. Add 1 ml glycoprotein solution to an opaque vial containing 5 mg Oxidation Reagent ($NaIO_4$) and swirl gently to dissolve the oxidizing agent.

3. Incubate the sample in the dark at room temperature for 30 minutes.

4. Stop the reaction and remove unreacted Oxidation Reagent by desalting and buffer exchange through Sephadex G-25 column.
Equilibrate a 5 ml Sephadex G-25 column with Coupling Buffer. Apply the oxidized sample to the column and allow it to enter the gel bed. Apply a 0.5 ml rinse of Coupling Buffer and allow it to enter the gel bed. Finally apply 2 ml Coupling Buffer and collect the eluent, which contains ideally 2.5 – 5.0 mg/ml oxidized glycoprotein.

Coupling:

1. Wash 1 ml **SiMAG-Hydrazide** particles (10 mg/ml) 2 x with 1 ml Coupling Buffer using the magnetic separator.

2. After the second wash step resuspend the magnetic particles in 0.75 ml Coupling Buffer. Mix the particles with 0.25 ml oxidized protein solution (2.5 – 5.0 mg/ml). This 1 ml suspension contains 0.625 – 1.25 mg oxidized protein and 10 mg SiMAG-Hydrazide beads. Incubate for a minimum of 6 hours at room temperature.

3. After incubation wash the particles 3 x with 1 ml Wash & Coupling Buffer.

4. Add 0.2 ml Blocking Buffer to the washed particles and mix gently for 30-60 minutes.

5. Wash the particles 10 x with 1 ml Storage Buffer.

6. Resuspend the particles in Storage Buffer.