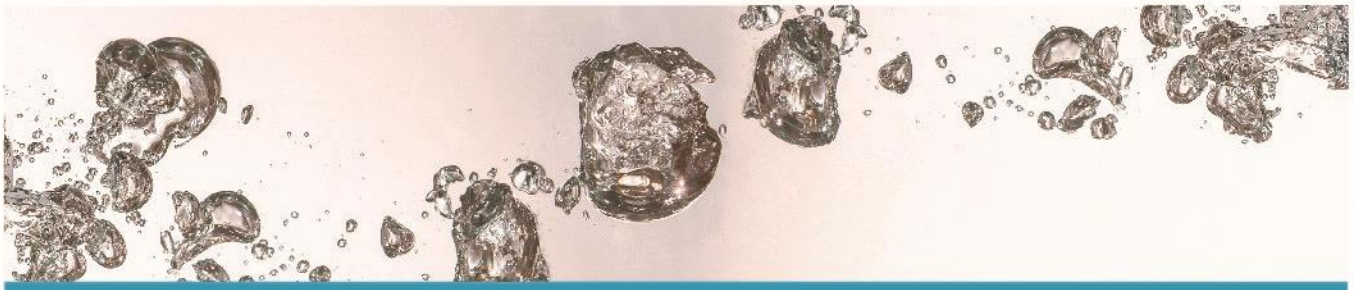
CIM Convective Interaction Media®

IMMOBILISATION PROCEDURE



For CIM® HDZ Monolithic Columns

This procedure applies to CIMac™ Analytical Columns (0.1 mL), CIMmic™, and CIMmultus™ Advanced Composite Columns (1 mL and larger) of hydrazide (HDZ) activation chemistry.

About CIM® HDZ activated monoliths

Hydrazide activated monolithic columns can be used to immobilise glycoproteins through oxidised sugar groups and other carbonyl containing molecules. Antibodies, in particular immunoglobulins G (IgG) containing abundant glycosylation, can be covalently bound to the matrix leaving unobstructed antigen binding sites. The resulting affinity columns provide high purification efficiency and no leaching of the immobilised ligand.

Before you begin

Before you begin with the immobilisation procedure, read the product's documentation supplied in the package.

Due to the structure of the monolithic polymer, some of the conditions needed for immobilisation may be different than with traditional chromatographic supports. Please consider the following when preparing a specific immobilisation procedure:

- **pH stability of the ligand**

Coupling reactions with hydrazide are performed at lower pH values (5 – 7.5) in suitable buffers. The pH range is further limited by the ligand stability which should be taken into account when performing an immobilisation. Glycoconjugates are increasingly stable at pH closer to neutral, therefore storage is recommended in a neutral pH buffer.

- **Thermal stability of the ligand**

The coupling of the ligand is achieved by a reaction between the HDZ groups of the support and carbonyl groups on the ligand. The coupling temperature should be based on thermal stability of the ligand. For IgG immobilisation, satisfactory results are achieved when the reaction takes place overnight at 20 °C (68 °F).

- **Composition of the coupling buffer**

The coupling buffer type significantly influences the coupling efficiency. In cases where the ligand stability depends on the environment the selection of the buffer is of utmost importance. Recommended buffer for IgG oxidation is phosphate buffered saline (PBS), while the buffer of choice for IgG immobilisation on CIM® HDZ support is 2-(N-morpholino) ethanesulfonic acid (MES). Optimisation of coupling conditions should be performed separately for other ligands.

- **Immobilisation protocol**

The choice of the coupling method depends on the properties of the ligand to be immobilised (the chemistry of its active groups, pH and temperature stability, reactivity, etc.). The following immobilisation procedure is an example of optimised coupling method for IgG and can be used as a platform process for different polyclonal or monoclonal IgGs.

Publication #: IMM-HDZ-1706

Immobilisation protocol for IgG

It is recommended to use an IgG sample of concentration 2 mg/mL in 10 mM phosphate, 0.1 M NaCl, pH 7.2 buffer. PBS buffer is also accepted. For an efficient immobilisation, between 3 and 10 mg of desired ligand should be available per mL of monolith.

Prepare the system and the column per guidelines in the Instruction manual. With compatible columns, a syringe can be used to perform the immobilisation.

Use a low flow rate for the immobilisation procedure, up to half of the maximum flow rate for the type of column used (see the PSIM for more information).

To immobilise the IgG, follow these steps:

1. Prepare the oxidation solution (20 mM sodium periodate ($NaIO_4$) dissolved in 10 mM phosphate, 0.1 M NaCl, pH 7.2 buffer). The solution is light-sensitive. It should be prepared shortly before immobilisation and kept in the dark. It takes several minutes to completely dissolve $NaIO_4$ in the buffer.
2. Mix the IgG sample of 2 mg/mL concentration with a solution of freshly prepared oxidation solution in a 1:1 (v:v) ratio and incubate for 30±5 min at 20 – 25 °C (68 – 77 °F). This will condition the sample for coupling to the hydrazide monolith by oxidising the glycosylated moieties of the antibody to aldehyde groups.
3. Quench the reaction by addition of ethylene glycol (25 µL of ethylene glycol per mL of solution).
4. Dilute the quenched reaction mixture 15-fold with 50 mM MES buffer, pH 5.2.
5. Filter the solution through 0.45 µm filter. This is the solution for immobilisation and it should be used at latest 1 h after preparation.
6. Flush the column with at least 10 column volumes (CV) of deionised water followed by 10 CV of 50 mM MES buffer, pH 5.2.
7. Pump the immobilisation solution through the monolithic column at a low flow rate (see PSIM for details).
8. Wash the column with 10 CV of 50 mM MES buffer, pH 5.2.
9. Seal the column with blind stoppers and keep at 20 – 25 °C (68 – 77 °F) for 15-24 hours.
10. Wash the column with at least 10 CV of 20 mM phosphate, 1 M NaCl, pH 7.2 or other neutral buffer containing 1 M NaCl.
11. Wash the column with at least 10 CV of PBS or other neutral buffer containing between 0.1 and 0.2 M NaCl.
12. Store the column in PBS, pH 7.2 containing 0.2 g/L sodium azide (NaN_3).

Note: The coupling bond formed between the support and protein is a hydrazone bond, which is considerably more stable than a Schiff base formed with a simple amine. The hydrazone bond is sufficiently stable for most applications. If desired, however, the double bond can be reduced to a more stable secondary amine bond using sodium cyanoborohydride.

Regeneration, cleaning in place and sanitisation procedures

Cleaning, regeneration and sanitisation procedures are ligand and application specific. To ensure the reusability of the column, specific procedures should be prepared to care for the immobilised column.

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Storage

Storage conditions should be determined based on the ligand stability. It is recommended to store the column between 2 °C (36 °F) and 8 °C (46 °F) in a suitable storage solution.

WARNING: Do not store the column below 0 °C (32 °F).
WARNING: Never let the monolith dry out!

Troubleshooting

1. **Quantity of immobilised ligand is small:**
   • Optimise the immobilisation procedure by considering the following parameters: buffer composition, time, pH, and temperature or ligand concentration.
   • Ensure that the monolithic column has been thoroughly flushed and equilibrated with the coupling buffer.
   • Prepare a new column and compare.

2. **Quantity of immobilised ligand is as expected, but affinity/capacity is low:**
   • Check ligand affinity before immobilisation. The ligand may be unstable, degraded or old.
   • The chromatographic binding and eluting buffers may not be optimal. Using an unsuitable buffer can damage the column.
   • Residual particles in the sample may be causing fouling of the column or blockage of pores through non-specific binding. Consider developing a procedure to sanitise the column or a CIP procedure.
   • If the immobilisation site is in the vicinity of the active site of the ligand, the latter may be sterically hindered. Consider using a different immobilisation strategy.

3. **Loss of binding/capacity with time:**
   • Affinity columns have a limited lifetime, especially when not used regularly.
   • Eluting conditions may not be optimal, i.e. either too weak for complete elution of the target molecule, or too strong and therefore damaging or modifying the immobilised ligand. Both causes lead to a loss of binding capacity.

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Additional reading

For additional information you may consult the following articles and application notes:

- App note: Versatility of CIM monoliths for covalent immobilisation of antibodies
- App note: A049 Immobilisation of proteins onto CIM – developing the most efficient affinity chromatographic monolith
- Vidic, Urška, et al. "Semi-high-throughput isolation and N-glycan analysis of human fibrinogen using monolithic supports bearing monoclonal anti-human fibrinogen antibodies." Electrophoresis (2017).
- Tarasova, Irina A., et al. "Depletion of human serum albumin in embryo culture media for in vitro fertilization using monolithic columns with immobilized antibodies." Electrophoresis 37.17-18 (2016): 2322-2327.
- Trbojević-Akmačić, Irena, et al. "Chromatographic Monoliths for High-Throughput Immunoaffinity Isolation of Transferrin from Human Plasma." Croatica Chemica Acta 89.2 (2016): 1-9.

For any additional information please contact us:

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