Sepharose Coating

Covalent immobilization of a molecule may result in higher signals than with adsorption coating, as well as reduce the amount of molecule necessary. If the molecule contains a free primary amine, NHS-activated Sepharose™ (4 Fast Flow, Catalog #17-0906-01, GE Healthcare) can be used. This particular activated sepharose incorporates a 15-carbon spacer arm that may reduce steric hindrance. The resulting coupling forms a stable, uncharged linkage that can help to minimize nonspecific binding (*Figure 1*).

1. Aliquot 1 mL of NHS-activated Sepharose into a microcentrifuge tube. Spin down the gel, there should be about 0.5 mL of visible gel in isopropanol. If there is enough gel, discard the supernatant. If not, then aliquot more sepharose into the tube until the gel reaches about 0.5 mL.

2. Quickly rinse the beads five times with cold dH_2O, spinning and discarding the supernatant each time.

3. Rinse once with a non-amine containing solution, typically 50 mM carbonate buffer, pH 9.0-9.6. Spin down and discard the supernatant.

4. Add 1 mL of the coating solution. This solution consists of 10-20 µg/mL molecule in a non-amine containing solution. Make sure the beads are fully suspended in solution.

5. Rock/tumble bead vials at room temperature for 2 hours or overnight at 4°C.

6. Allow the beads to settle or pulse centrifuge at a low speed to pellet the beads. Discard the supernatant without disturbing the settled beads.

7. Add 1 mL of blocking solution. This solution consists of 10 mg/mL BSA in 1 M Tris buffer, pH 8.0-8.5. Make sure the beads are re-suspended in blocking solution.

8. Rock/tumble bead vials at room temperature for 1 hour or overnight at 4°C.

9. Store in blocking solution at 4°C until ready for use.

Notes:

- Be sure to use the "Soft Bead Handling" template for timing setup of the beads. Keep all flow rates at or below 1 mL/min in the sample timing.
- Larger sample volumes (greater than 4 ml) should be avoided, as the flow cell screen will clog more rapidly.
- Coupling is more efficient at higher pH (9.6), provided that the protein to be coupled can withstand these conditions.
- Using cold buffer in place of cold dH_2O for rinsing is not recommended because buffer salts may precipitate when mixing with the bead slurry solvent (isopropanol).
- Amine-containing buffers (Tris, glycine, or BSA buffers) must not be used in the coupling step because their amine functional groups will compete with the protein for coupling sites.
- Using similar protocols, our customers also have reported successfully using Reacti-Gel® (6X agarose, Catalog #20259, Pierce Biotechnology) and CNBr-activated sepharose (4 Fast Flow, Catalog #17-0981-01, GE Healthcare).

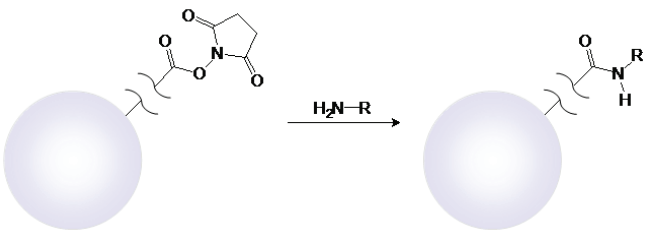

Figure 1. Covalent immobilization chemistry on NHS-activated sepharose beads. *The terminal carboxyl group of a linker arm on a sepharose bead is activated by eserification with N-hydroxysuccinimide. This active ester reacts with primary amines to form highly stable amide bonds, coupling the molecule to the bead.*