

CNBr-Sepharose Beads

Notes

- i) Coupling occurs through Lysine
- ii) the binding is protein concentration dependent, but above ~20 mg/ml binding quickly falls off
- iii) pH for binding is best ~ 8 – 10 but may be done at a lower pH. At pH 5 binding is 3 – 10 fold less than at pH 8.0
- iv) If there is too much binding you can get steric hindrance of the binding sites
- v) The salt can also be reduced, it all depends on the protein you want to couple. but again binding may be reduced
- vi) ethanolamine introduces a few charged groups with the gel. The effect of these changes on non-specific adsorption of proteins owing to their poly-electrolyte nature is overcome by the 0.5M NaCl
- vii) The protein should be in 0.1 M Na HCO₃, 0.5 M NaCl pH 8.3. **AVOID TRIS**

Reagents

Coupling buffer: 0.1M NaHCO₃, 0.5M NaCl pH 8.3

Wash buffer: 0.1M acetate, 0.5M NaCl pH 4.0

1 mM HCl

1 M ethanolamine-HCl pH 9 (stock is 16.7 M)

Method

1. Use 1 g CNBr-Sepharose per 3.5 ml bead volume required. Swell for 15 min in 1mM HCl. Wash with 50 - 100 ml PBS
2. Wash on sintered glass filter with 1 mM HCl (200 ml/g dry beads). This washes out the dextran and lactose used as stabilizers, and preserves the active groups which hydrolyse at high pH.
3. Wash with coupling buffer (5 ml/g dry beads), move quickly onto next step as the pH is high.
4. Use 5 – 10 mg protein for 1 ml swelled resin in the ratio of 1 resin: 2 protein v/v. Mix with gentle tumbling at room temperature for 2 hours or at 4°C overnight.
5. Spin 500 x g, 2 minutes. Measure A₂₈₀ of supernatant to check coupling (should be > 90%).
6. Add equal volume 1 M ethanolamine-HCl pH 9 to block remaining active groups: Roll at 4°C for 40 minutes.
7. Wash out the ethanolamine in coupling buffer (use the Büchner flask and sintered funnel)
8. Wash 4 – 5 times alternately in coupling buffer (pH 8.3) and wash buffer (pH 4.0). This ensures that no free ligand remains ionically bound to the immobilized ligand. It does not remove covalently-bound protein.
9. Store for months at 4°C in PBS + 0.1% azide. The azide must be washed out before use. The protein is stabilized on the column and stores better than the free protein.

Antibody Purification

1. Mix 1 ml (i.e. 2 ml 50% slurry) beads with 10 ml PBS and 10 ml crude antisera, 4°C overnight
2. Wash with 50 - 100 ml PBS
3. Elute in 6 x 1 ml 200 mM glycine pH 2.8, collecting into 100 µl 1 M Tris-Cl pH ~8.5
4. Test each fraction by dot-blot; combine high-activity fractions and freeze in aliquots. Dialyse against (e.g.) T/PBS if you like, but it's not necessary.