

## AGAROSE BEAD ACTIVATION PROTOCOL

**To form formyl coupling groups on supports containing no diols (p76).** *The glycidol introduces adjacent hydroxyls that are then oxidized by NaIO<sub>4</sub>.*

1. Equipment necessary: 5 mL of beads, 1 M NaOH, NaBH<sub>4</sub>, glycidol, 1 M NaCl, water, vacuum filtration, 2 x 25 mL Erlenmeyer,
2. To 5 mL of beads wash extensively with water to remove sodium azide.
3. Wash with 200 mL of 1 M NaOH and place in first Erlenmeyer.
4. Weigh out 10 mg of NaBH<sub>4</sub> into another 25 mL Erlenmeyer. Add 10 mL 1 M NaOH.
5. Suspend gel in 5 mL of solution prepared in step 4.
6. Add 500 µL glycidol while stirring. Allow reaction to continue overnight.
7. Wash extensively with water, 1 M NaCl, and water again.

**Periodate Oxidation (p 75).** *Cleaves carbon bond on two adjacent secondary hydroxyls and creates two terminal aldehyde groups.*

1. Equipment necessary: 0.2 M NaIO<sub>4</sub> (protect from light, MW 213.91), water, 25 mL Erlenmeyer, 0.02% sodium azide (0.2 g/L), 5 mL beads.
2. Add wet gel cake to 5 mL of 0.2 M NaIO<sub>4</sub> (42.8 g/L). Cover with aluminum foil.
3. Mix and allow reaction to continue for 90 minutes (no longer)
4. Add 0.1 mL of glycerol per mL of reaction solution ~ 1 mL.
5. Store in 0.02% NaN<sub>3</sub>.

**Coupling of Avidin or Streptavidin (p 200).** *NaCNBH<sub>3</sub> couples amine containing avidin by secondary amine linkage.*

1. Equipment necessary: 0.1 M sodium phosphate buffer (pH 7.0), sintered glass funnel, avidin or streptavidin, NaCNBH<sub>3</sub>, 50 mL Erlenmeyer, water, 1.0 M NaCl, beads.
2. Wash 2 mL of settled beads with 100 mL of 0.1 M sodium phosphate buffer (pH 7.0) in a sintered glass funnel and suction dry to a moist cake.
3. Add the gel to a 2-4 mg/mL streptavidin or avidin solution (in 0.1 M sodium phosphate buffer pH 7.0).
4. Add 12 mg NaCNBH<sub>3</sub> and mix overnight at room temperature in a 50 mL Erlenmeyer. Wash with 200 mL of water, 200 mL of 1.0 M NaCl, and 200 mL of water.

**Blocking Excess Aldehydes (p 200).** *Tris contains a primary amine that readily reacts with aldehydes in the presence of a reductant to form unreactive hydroxyls.*

1. Equipment necessary: 1.0 M Tris-HCL, pH 7.4, NaCNBH<sub>3</sub>, 25 mL Erlenmeyer, 1 M NaCl, Water, 0.02% sodium azide.
2. Suspend gel in 2 mL of 1.0 M Tris-HCL pH 7.4, containing 12 mg of NaCNBH<sub>3</sub> and mix gently for 1 hour. (10 µL of 5 M cyanoborohydride in 1 M NaOH per mL of solution).
3. OR: Adjust pH of reaction mixture to 9.0 by adding 1 M NaOH. Add NaBH<sub>4</sub> (2.5 mg) and mix for 30 minutes.
4. Rinse gel with 200 mL of water, 200 mL of 1.0 M NaCl, and 200 mL water.
5. Store in 0.02% sodium azide.