



## Immobilized Steroid Beads

### What are they?

Sepharose™ 4 fast flow (FF) beads covalently modified with steroids or other ligands such that ligand binding to receptors is not compromised resulting in high receptor-binding specificity. These beads feature a spacer arm and a high flow rate.

### What can they be used for?

- Efficient isolation and purification of receptor proteins (nuclear and others)
- Ligand binding affinity studies
- Preparation of receptor-ligand complexes for crystallography studies.
- Assisting in the structure-based design of receptor selective ligands (e.g. see Manas et al.)

### How do they work?

Beads can be added “batchwise” to the crude protein extract and then packed into a glass column and unbound material eluted away by gravity flow. Alternatively, the beads can be pre-packed into a column and the crude receptor protein mixture applied to the column for purification. After elution of unbound material, the bound receptor is eluted by treating the beads with a solution of a ligand with comparable or greater affinity for the receptor. The final step is usually size exclusion chromatography to remove excess ligand from the protein.

### FAQs

**Can the beads be reused?** Yes, see reference Salman et al. for a procedure for nortestosterone Sepharose bead. This procedure should also be applicable for the dexamethasone Sepharose bead. In the case of estradiol and androstan beads these can be cleaned by washing first with 4 - 6 M urea in tris buffer (pH around 8) and then following the same procedure for nortestosterone Sepharose. All beads should be stored in 20% aqueous ethanol at 4 - 8° C until further use.

**Why is carboxymethylation with iodoacetic acid sometimes performed before eluting the protein from the column?** To derivatize exposed cysteines in the protein and prevent formation of cross-linked dimer artifacts. This is not necessary however if the protein is stored with a reducing agent. See reference, Hegy et al.

### How much receptor can be bound to the bead?

This depends upon the structure of the receptor of interest. As a guide, it is known that ~ 7 mgs of ER (MW ~30,000) can be bound and eluted from 1.0 mL of the estradiol Sepharose beads.

### How can non-specific binding of proteins to the beads be reduced?

One option is to coat the bead with bovine serum albumin (BSA). Try both defatted and non-defatted BSA using a concentration of 0.1-0.2 mg/mL in buffer for washing the bead. Finally, wash with buffer containing salt to remove unbound BSA. Another option for blocking non-specific binding might be to treat the Sepharose with 0.2 M ethanolamine at pH 10 and 4 ° C according to the procedure of Williams et al.

### References:

Williams AJK et al. Non-specific binding to protein A Sepharose and protein G Sepharose in insulin autoantibody assays may be reduced by pre-treatment with glycine or ethanolamine. *J. Immunol. Methods*, **314**:170-173 (2006).

Manas ES et al. Structure-based Design of Estrogen Receptor-Beta Selective Ligands. *J. Am. Chem. Soc.*, **126**, 15106-15119 (2004).

Hegy GB et al. Carboxymethylation of the human estrogen receptor ligand-binding domain-estradiol complex: HPLC/ESMS peptide mapping shows that cysteine 447 does not react with iodoacetic acid. *Steroids*, **61**:367-373 (1996).

Salman M et al. A Progesterone Receptor Affinity Chromatography Reagent: 17 $\alpha$ -Hexynyl Nortestosterone Sepharose. *J Steroid Biochem.*, **26**, 3, 383-391(1987).

