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Sepharose® 6B Immobilized Steroid Beads

Affinity chromatography beads covalently modified with steroids or other ligands such that ligand binding to receptors is not compromised resulting in high receptor-binding specificity.

What can they be used for?

- Ligand binding affinity studies
- Efficient isolation and purification of receptor proteins (nuclear and others)
- 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 Salman et al. reference 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 4-6M 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 4-8ºC until further use.

Why is carboxymethylation with iodoacetic acid sometimes performed before eluting the protein from the column?
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 Hegy et al. reference.

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 ~7mg of ER (MW ~30,000) can be bound eluted from 1.0mL 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 0.1-0.2mg/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 0.2M ethanolamine at pH 10 and 4°C according to the procedure. See Williams et al. reference.

Ordering Info

Cat. #	Product	Typical ligand loading
24858	Androstan® 6B	10-14 µmoles/mL bead
24859	Dexamethasone Sepharose® 6B	10-14 µmoles/mL bead
24861	Estradiol Sepharose® 6B	10-14 µmoles/mL bead
24860	Nortestosterone Sepharose® 6B	10-14µmoles/mL bead
24869	Long Spacer Arm Dexamethasone Sepharose® 6B	10-14 µmoles/mL bead
24868	RHC-80267 (U-57908) Sepharose® 6B	12-15 µmoles/mL bead

References:

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

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

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).

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).

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