Product Information

Blue Sepharose® CL-6B

Catalog Number R8752
Storage Temperature 2-8 °C

Product Description
Isolation of albumin from whole human plasma using Blue Sepharose has been described. Blue Sepharose has been used in the binding of albumin from different species to assess the binding constant of the protein to the gel. Albumins both in isolated form and in sera can bind to the resin. Other serum proteins including lipoproteins are also adsorbed to the resin, which indicates an additional chromatographic step, such as gel filtration, may be necessary to obtain purified albumin from serum. The equilibration buffer typically used to bind albumin is 50 mM Tris-HCl, pH 8.0, containing 50 mM sodium chloride. Albumins can be eluted using the equilibration buffer with 200 mM sodium thiocyanate. Human albumin possesses the highest affinity for the dye, rat albumin the lowest (K_a of 3.9 x 10^4 M^-1 and 1.7 x 10^4 M^-1, respectively).

Blue Sepharose has been used in the purification of α-T and β-γ-T subunit complexes of holo-transducin, a GTP binding protein in the visual system involving rhodopsin. Concentrated holo-transducin was applied to a 50 ml Blue Sepharose column equilibrated with 10 mM HEPES, pH 7.5, with 6 mM MgCl_2, 1 mM DTT, and 25% glycerol. The β-γ-T subunit complex was eluted from this column by washing with 200 ml of the same buffer. The α-T subunit was then eluted from the resin with the same buffer containing 0.5 M KCl. The α-T subunit was tightly bound to GDP (formed from hydrolysis of GTP under conditions used to elute the holo-transducin from rhodopsin). This product has also been used in the purification of NAD^+ and NADP^+ dependent enzymes, such as alcohol dehydrogenase. The cofactor which has greatest affinity for the enzyme is most effective in the elution of the enzyme.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The rehydrated resin can be stored in water at 2-8 °C during continuous use. For longer-term storage, equilibrate the resin in 20% ethanol and store at 2-8 °C.

Procedure
Preparation Of The Gel
The required amount of Blue-Sepharose powder is swollen for 15 minutes in water and washed on a sintered glass filter with distilled water. For washing, approximately 200 ml of water is used, added in aliquots, for each gram of dry powder. One gram swells to approximately 3.5 ml.

Elution
Specifically-bound proteins are eluted with low concentrations of the specific cofactor added to the buffer. Either pulsed elution or a continuous gradient can be used, with elution of the enzyme in the range of 1-20 mM. Change of pH or ionic strength of the buffer can also be used to elute proteins (up to 1 M NaCl concentration for enzyme elution). Less specifically-bound proteins, such as albumin or interferon, require more severe eluents such as urea or potassium isothiocyanate.

Cleansing
Wash with 4-5 washing cycles of alternate high and low pH. Strongly bound material such as lipoproteins may be removed with chaotropic or dissociating agents such as 6 M urea.

References

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