

Depletion of Abundant HSA from Serum

INTRODUCTION

Highly abundant serum proteins, such as albumin and immunoglobulins, are one of the major obstacles in proteomic analysis of serum or plasma samples. The dynamic range of concentration of proteins present in serum samples makes it necessary to remove these proteins to allow detection and analysis of less abundant serum proteins. Depletion of the most abundant proteins in human serum will significantly increase the sensitivity in analyses of the remaining proteins.

The Anti-HSA Affibody[®] molecule is a highly specific affinity ligand, well suited for depletion of HSA from human serum. Single point attachment ensures high capacity and reproducible immobilization, and the high stability of the Affibody[®] molecule allow at least 300 times re-use without loss of binding capacity.

RESULTS

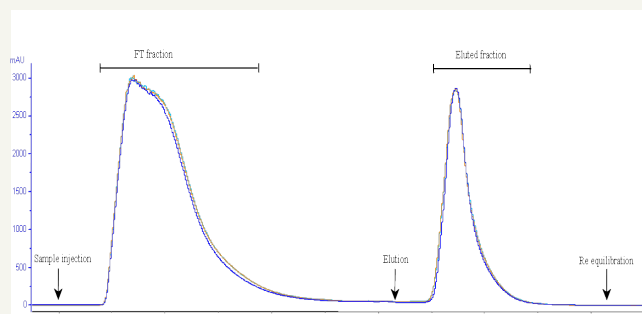


Fig. 1. Overlay of chromatograms from run number 1, 50 and 300 of the repeated affinity removal of HSA from serum. The depletion procedure can be reproducibly repeated at least 300 times without loss of binding capacity.

Overlay chromatograms of repeated affinity removal of HSA from serum are shown in figure 1. The chromatograms represent run number 1, 50 and 300 after consecutive injections of 300 μ l of five times diluted human serum on 0.37 ml SulfoLink[®] Coupling Gel with immobilized Anti-HSA Affibody[®] molecule. The peak area of the eluted fraction after each run is plotted in figure 2. The identical chromatograms and consistent peak areas of eluted fractions prove that the depletion procedure can be reproducibly repeated at least 300 times without loss of binding capacity. SDS-PAGE analysis of flow-through fractions and eluted fractions shown in figure 3 demonstrate that the high specificity of the Anti-

HSA Affibody[®] molecule is maintained through all the 300 consecutive injections. The capacity of this affinity matrix allows for depletion of HSA from 800 μ l of five times diluted human serum per ml matrix corresponding to 160 μ l of undiluted human serum per ml matrix.

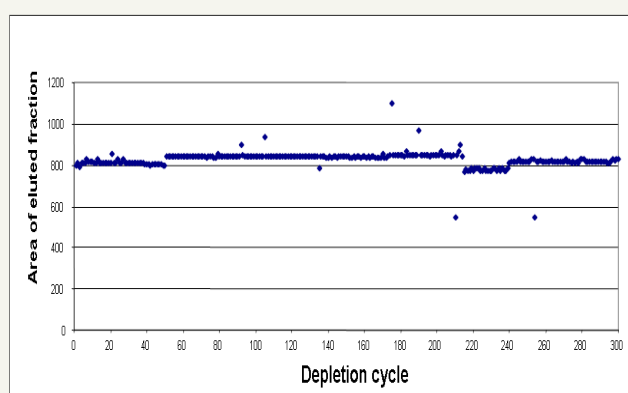


Fig. 2. Peak area of the eluted fraction after each run of affinity removal. The consistent peak area proves that the depletion procedure can be reproducibly repeated at least 300 times without loss of binding capacity.

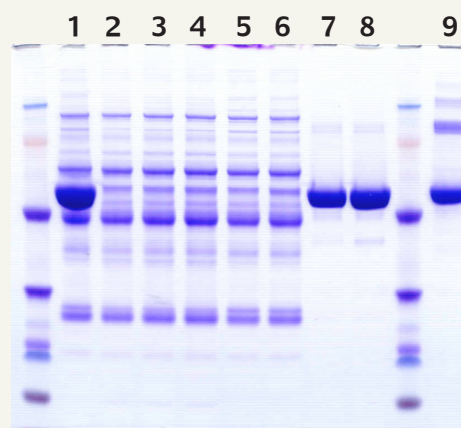


Fig. 3. SDS-PAGE analysis of flow-through fractions (FT) and eluted fractions after repeated affinity removal of HSA from serum. Lane 1: Untreated 5x diluted serum sample; lane 2: FT run 1; lane 3: FT run 75; lane 4: FT run 150; lane 5: FT run 225; lane 6: FT run 300; lane 7: eluate run 1; lane 8: eluate run 300; lane 9: HSA standard.

Depletion of Abundant HSA from Serum

MATERIALS AND BUFFERS

Sample: Human serum (PAA Laboratories GmbH cat no C11-061), diluted five times in running buffer.

Column: SulfoLink[®] Coupling Gel (Pierce cat no 20401) with immobilized Anti-HSA Affibody[®] molecule packed in a Tricorn 5/20 column (GE Healthcare), column volume 0.37 ml.

Chromatography system: ÄKTAexplorer 10S (GE Healthcare)

Running buffer: 25 mM Tris-HCl, 1 mM EDTA, 200 mM NaCl, 0.05 % Tween 20, pH 8.0

Elution buffer: 0.5 M Acetic acid, 1 M Urea, 100 mM NaCl, pH 2.8

EXPERIMENTAL PROCEDURE

For immobilization of Affibody[®] molecules onto SulfoLink[®] Coupling Gel from Pierce, see separate protocol.

A human serum sample was centrifuged at 3,000 x *g* for 5 minutes. The supernatant was diluted five times in running buffer and filtered through a 0.45 µm filter. Chromatography was run on an ÄKTA Explorer 10S instrument at a flow rate of 0.2 ml/minute. The absorbance at 280 nm was monitored.

300 µl of the diluted serum was applied to a column containing 0.37 ml of SulfoLink[®] Coupling Gel coupled with 3 mg Anti-HSA Affibody[®] molecule per ml SulfoLink[®] Coupling Gel followed by washing with 6.5 column volumes of running buffer. The flowthrough fraction was collected between 0.4 and 1.9 ml after sample injection. The bound fraction was eluted with 6.5 column volumes of elution buffer and the eluted fraction was collected between 0.7 and 1.7 ml after start of elution. The column was re-equilibrated with 5 column volumes of running buffer between each run.

Immobilization of Affibody[®] Molecules to SulfoLink[®] Coupling Gel

MATERIALS AND BUFFERS REQUIRED

Matrix: SulfoLink[®] Coupling Gel (Pierce cat no 20401)

Desalting column: PD-10 Desalting Column (GE Healthcare cat no 17-0851-01)

Dithiothreitol (DTT) stock solution: Prepare a 0.5 M stock solution by dissolving DTT in water.

Affinity ligand: Affibody[®] molecule with a unique C-terminal cysteine

Coupling buffer: 50 mM Tris-HCl, 5 mM EDTA, pH 8.0

Blocking buffer: 50 mM L-cysteine in coupling buffer

Wash solution: 1 M NaCl

Storage buffer: Phosphate buffered saline (PBS) containing 0.02% sodium azide (NaN₃)

REDUCTION OF THE C-TERMINAL CYSTEINE OF THE AFFIBODY[®] MOLECULE

1. Dissolve 1-3 mg of the Affibody[®] molecule in 2 ml coupling buffer. To reduce the unique C-terminal cysteine, add 80 µl of 0.5 M DTT to the Affibody[®] solution to a final concentration of 20 mM DTT. Incubate at room temperature for 1-2 hours.
2. Exchange the buffer using a PD-10 column: equilibrate the column with 25 ml coupling buffer, apply the sample (2 ml) and allow it to enter the bed completely and then add another 0.5 ml coupling buffer. Elute with 3 ml of coupling buffer. **Note:** This is a modification of the original protocol for PD-10 columns.
3. Continue with immobilization immediately after buffer exchange to avoid re-oxidation.

COUPLING AFFIBODY[®] MOLECULE TO MATRIX

1. Equilibrate SulfoLink[®] Coupling Gel to room temperature and add 2 ml of slurry (1 ml packed matrix) to an empty disposable column.
2. Equilibrate with 4 ml of Coupling Buffer. **Note:** Throughout the entire procedure, do not allow the bed to run dry; instead add additional solution or replace the bottom cap on the column whenever the buffer drains down to the top of the bed.
3. Replace the bottom cap on the column and add the reduced sample. Retain a small sample of the protein solution for later determination of coupling efficiency.

4. Replace the top cap and mix by rocking or end-over-end mixing at room temperature for 1 hour.
5. Sequentially remove top and bottom column caps and allow the solution to drain from the column into a clean tube.
6. Place the column over a new collection tube and wash with 3 ml of coupling buffer.
7. Determine the coupling efficiency by comparing the protein concentrations (e.g., by absorbance at 280 nm) of the unbound fraction (Step 5) to the starting sample (Step 3).

BLOCKING NONSPECIFIC BINDING SITES

1. Replace the bottom cap on the column.
2. Apply 1 ml of blocking buffer.
3. Replace the top cap and mix for 1 hour at room temperature.

WASHING AND STORAGE

1. Sequentially remove the top and bottom caps and allow the buffer to drain from the column.
2. Wash with at least 6 ml of wash solution.
3. Wash with 2 ml of storage buffer.
4. Replace the bottom cap and add an additional 1 ml of storage buffer.
5. The ligand is now covalently coupled to the support through its sulfhydryl groups and can be used for affinity purification. Replace the top cap and store the column upright at 4°C.

LIMITATIONS

Warranty: Affibody[®] products are warranted to meet stated product specifications and to conform to label descriptions when used and stored properly. Unless otherwise stated, this warranty is limited to one year from date of sales for products used, handled and stored according to Affibody AB's instructions. Affibody AB's sole liability is limited to replacement of the product or refund of the purchase price. Affibody[®] products are supplied for research use only. They are not intended for medicinal, diagnostic or therapeutic use. Affibody[®] products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from Affibody AB.

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