

## Depletion of Abundant IgG in 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-IgG Affibody<sup>®</sup> molecule is a highly specific affinity ligand, well suited for depletion of IgG from human serum. Single point attachment ensures high capacity and reproducible immobilization, and the high stability of the Affibody<sup>®</sup> molecule allow at least 300 times re-use without loss of binding capacity. embedded tissue sections is not recommended.

### RESULTS



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

Overlay chromatograms of repeated affinity removal of IgG from serum are shown in figure 1. The chromatograms represent run number 1, 50 and 300 after consecutive injections of 700  $\mu$ l of five times diluted human serum on 0.37 ml SulfoLink<sup>®</sup> Coupling Gel with immobilized Anti-IgG Affibody<sup>®</sup> molecule. The peak area of 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-IgG Affibody<sup>®</sup> molecule is maintained through all the 300 consecutive injections.

The capacity of this coupling gel allows for depletion of IgG from 1900  $\mu$ l of five times diluted human serum per ml gel, corresponding to 380  $\mu$ l of undiluted human serum per ml gel.

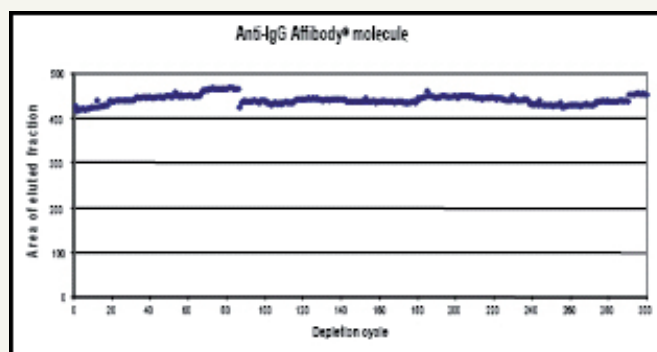


Fig. 2. Peak area of the eluted fraction after each run of affinity removal. The consistent peak area prove that the depletion procedure can be reproducibly repeated at least 300 times without loss of binding capacity.

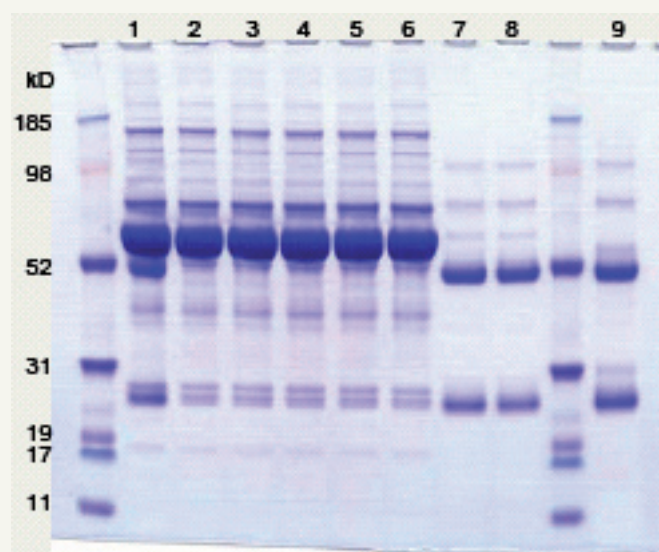


Fig. 3. SDS-PAGE analysis of flow-through fractions (FT) and eluted fractions after repeated affinity removal of IgG from human serum. Lane 1: Untreated 5x diluted serum sample; lane 2: FT run 1; lane 3: FT run 75; lane 4: FT run 150; lane 5: FT run 225; lane 6: FT run 300; lane 7: eluate run 1; lane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

## Depletion of Abundant IgG in Serum

### MATERIALS AND BUFFERS REQUIRED

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

**Column:** SulfoLink® Coupling Gel (Pierce) with immobilized Anti-IgG Affibody® molecule packed in a Tricorn 5/20 column (GE Healthcare), column volume 0.37 ml.

**Chromatography system:** Äkta Explorer 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

### 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. 700 µl of the diluted serum was applied to a column containing 0.37 ml of SulfoLink® Coupling Gel coupled with 3 mg Anti-IgG Affibody® molecule per ml SulfoLink® Coupling Gel and followed by washing with 30 column volumes of running buffer. The flowthrough fraction was collected between 0.4 and 2.3 ml after sample injection. The bound fraction was eluted with 7 column volumes of elution buffer and the eluted fraction was collected between 0.5 and 1.2 ml after start of elution. The column was re-equilibrated with 6 column volumes of running buffer between each run.

# Immobilization of Affibody<sup>®</sup> Molecules to SulfoLink<sup>®</sup> Coupling Gel

## MATERIALS AND BUFFERS REQUIRED

- SulfoLink<sup>®</sup> Coupling Gel (Pierce cat no 20401)
- PD-10 Desalting Column (GE Healthcare cat no 17-0851-01)
- Coupling buffer; 50 mM Tris-HCl, 5 mM EDTA, pH 8.0
- Dithiothreitol (DTT) stock solution. Prepare a 0.5 M stock solution by dissolving DTT in water.
- Affibody<sup>®</sup> molecule with a unique C-terminal cysteine

## PROTOCOL

### PROCEDURE FOR REDUCTION OF THE CYSTEINE RESIDUES:

1. Prepare a 5 mg/ml solution of the Affibody<sup>®</sup> molecule in coupling buffer. To reduce the unique C-terminal cysteine, add 40 µl of 0.5 M DTT per ml Affibody<sup>®</sup> protein solution to a final concentration of 20 mM DTT and incubate at room temperature for 2 hours.
2. Exchange the buffer using a PD-10 column: equilibrate the column with 25 ml coupling buffer, apply the sample followed by coupling buffer to a total volume of 2 ml, add another 0.5 ml coupling buffer. Elute with 3 ml of coupling buffer. Note that this is a modification of the original protocol for PD-10 columns.
3. Use immediately for coupling.

### PROCEDURE FOR IMMOBILIZATION:

Immobilize the reduced Affibody<sup>®</sup> molecule onto SulfoLink<sup>®</sup> Coupling Gel according to the recommendations from manufacturer. The depletion example presented in this application is performed using SulfoLink<sup>®</sup> Coupling Gel with a ligand density of 3 mg Affibody<sup>®</sup> molecule per ml gel. Note that it may be necessary to use more than 1 ml protein solution/ml coupling gel.

## LIMITATIONS

Warranty: Affibody<sup>®</sup> 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<sup>®</sup> products are supplied for research use only. They are not intended for medicinal, diagnostic or therapeutic use. Affibody<sup>®</sup> 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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