

## Insulin Capture from Human Serum

### INTRODUCTION

In laboratory scale, affinity chromatography is an excellent protein purification method. By immobilizing an affinity ligand onto a matrix, a protein of choice can be purified to high yield in a single step from a crude starting sample.

The Anti-Insulin Affibody<sup>®</sup> molecule is an ideal affinity ligand for chromatography applications. The molecule has a unique C-terminal cysteine for directed single-point chemical modification, facilitating immobilization and conjugation.

In order to establish the performance of the Anti-Insulin Affibody<sup>®</sup> molecule, a capture assay to purify insulin from human serum was developed.

### RESULTS

To demonstrate the binding capacity and specificity of the Anti-Insulin Affibody<sup>®</sup> molecule, 1.5 ml of five times diluted human serum spiked with insulin was injected on a column with 0.4 ml SulfoLink<sup>®</sup> Coupling gel with immobilized Anti-Insulin Affibody<sup>®</sup> molecule. The chromatogram of the purification is shown in figure 1. Eluted and flow-through fractions were analyzed by an SDS-PAGE analysis, shown in figure 2.

The analysis demonstrate the high specificity of the Anti-Insulin Affibody<sup>®</sup> molecule. Insulin present in the serum sample is not visible in the flow-through fraction and in the eluted fraction, only pure insulin can be detected. The results presented show that insulin can be removed or purified from a complex mixture of proteins. The binding capacity was determined to be approximately 1.8 mg human insulin per ml Anti-Insulin Affibody<sup>®</sup> coupled gel.

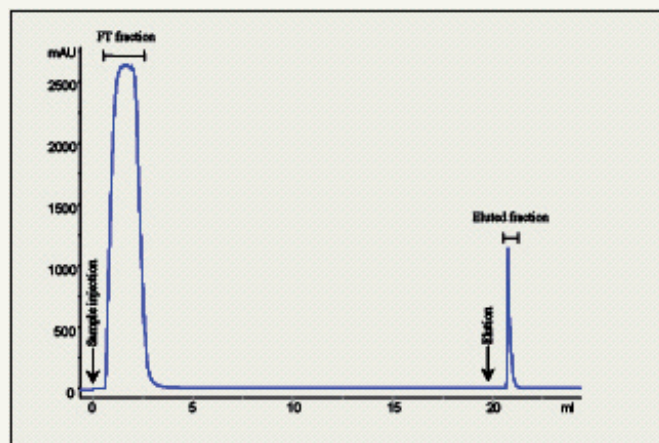


Fig. 1. Chromatogram showing the purification of insulin from human serum spiked with insulin on a column with immobilized Anti-Insulin Affibody<sup>®</sup> molecule.

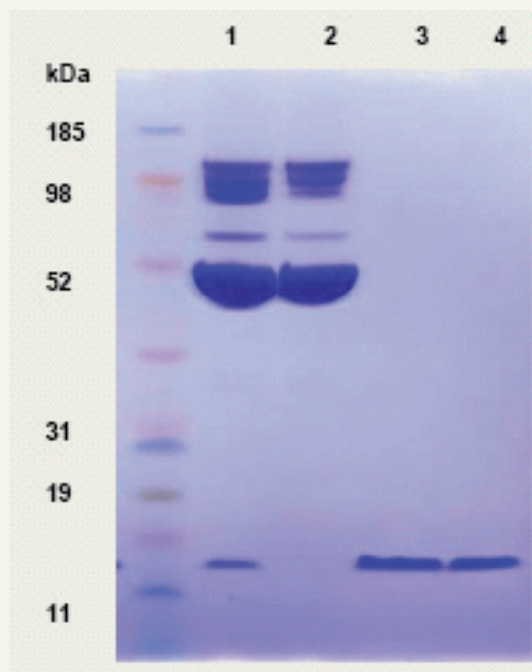


Fig. 2. SDS-PAGE analysis of injected, eluted and flowthrough fractions from the purification of insulin on a column with immobilized Anti-Insulin Affibody<sup>®</sup> molecule. Lane 1: human serum spiked with insulin; lane 2: flow-through fraction; lane 3: eluted fraction; lane 4: human insulin standard.

## Insulin Capture from Human Serum

### MATERIALS AND BUFFERS REQUIRED

**Sample:** Insulin spiked human serum: 0.5 mg/ml human insulin (Roche cat no 1376 497) in human serum (PAA Laboratories GmbH cat no C11-061) diluted 5 times in running buffer.

**Column:** SulfoLink® Coupling Gel with immobilized Anti-Insulin Affibody® molecule was packed in a Tricorn 5/20 column (GE Healthcare), the column volume was 0.4 ml.

**Chromatography system:** Äkta Explorer 10S (GE Healthcare)

**Running buffer:** PBST (2.68 mM KCl, 137 mM NaCl, 1.47 mM  $\text{KH}_2\text{PO}_4$ , 8.1 mM  $\text{Na}_2\text{HPO}_4$ , 0.05% Tween 20 pH, 7.4)

**Elution buffer:** 0.5 M Acetic acid, pH 2.8

### PROCEDURE

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

The gel was packed in a column which was connected to a chromatography system. 1.5 ml of insulin spiked human serum sample was applied to the column at a flow rate of 0.2 ml/minute, followed by washing with 40 column volumes of running buffer at a flow rate of 0.5 ml/minute. The binding capacity was determined to be approximately 1.8 mg human insulin per ml Anti-Insulin Affibody® coupled gel. Bound protein was eluted with 10 column volumes of elution buffer at a flow rate of 0.5 ml/minute. The absorbance at 280 nm was monitored. Flow-through fractions and eluted fractions were collected and analyzed by SDS-PAGE (Invitrogen NuPAGE 4-12% Bis-Tris Gel 1.0 mm X 10 well).

## 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 2 mg/ml solution of the Affibody<sup>®</sup> molecule in coupling buffer. To reduce the unique C-terminal cysteine, add 40  $\mu$ l 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 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 the manufacturer. The 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.

Affibody AB, P.O. Box 20137, SE-161 02 Bromma, Sweden. Phone: +46-8-598 838 00, Fax: +46-8-598 838 01, E-mail: [biotechnology@affibody.com](mailto:biotechnology@affibody.com), Web: [www.affibody.com/shop](http://www.affibody.com/shop).

Rev 070330