

Depletion of Transferrin from Human Plasma

INTRODUCTION

The presence of highly abundant proteins is one of the major obstacles in proteomic analysis of plasma samples. A pre-fractionation step, involving depletion of abundant proteins before protein profiling, facilitates the discovery and detection of less abundant plasma proteins. Today, the vast majority of commercially available depletion kits utilize antibodies as the affinity capture ligand for the removal of such proteins.

Here, we present the use of the small, highly specific and robust Anti-Transferrin Affibody[®] molecule for depletion of transferrin from human plasma. This study demonstrates that an affinity matrix based on the Anti-Transferrin Affibody[®] molecules has superior capacity and is an attractive and affordable alternative to other affinity ligands.

RESULTS

The Anti-Transferrin Affibody[®] molecule was single point immobilized, to a ligand density of 3 mg per ml matrix, through its C-terminal cysteine. To investigate the capacity of the affinity matrix, different volumes of five times diluted human plasma was loaded on a column packed with 0.2 ml Affibody[®] matrix. A typical chromatogram of a depletion procedure is shown in figure 1.

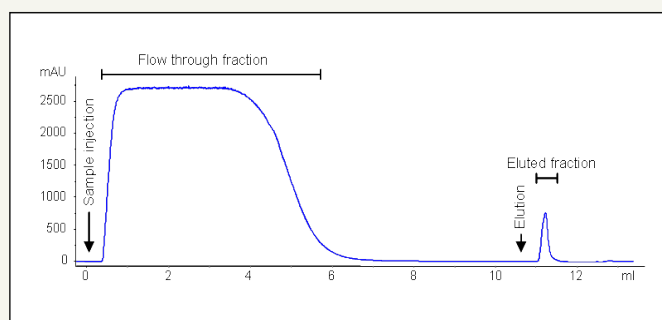


Fig. 1. Chromatogram showing depletion of transferrin from 4 ml of 5 times diluted human plasma on the column packed with 0.2 ml Anti-Transferrin Affibody[®] matrix.

The amount of transferrin was determined in loaded sample and flow through fractions using ELISA and achieved depletion was calculated (table 1). SDS-PAGE analysis of one of the eluted fractions is shown in figure 2.

ELISA analysis showed high depletion efficiency at high column load. 99.8 % transferrin depletion was achieved when a volume corresponding to 4 ml of undiluted human plasma was loaded per ml Anti-Transferrin Affibody[®] matrix. The SDS-PAGE analysis demonstrates that the Anti-Transferrin Affibody[®] molecule binds transferrin in plasma with high specificity.

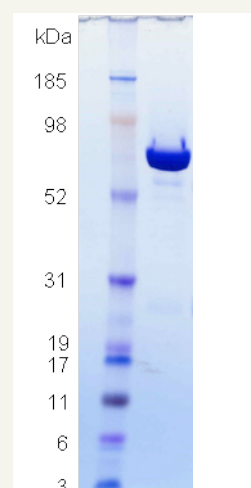


Fig. 2. SDS-PAGE analysis of eluted fraction from depletion of transferrin on a column with immobilized Anti-Transferrin Affibody[®] molecules. The analysis was performed at reduced conditions.

Volume plasma loaded on 0.2 ml column (µl)	Column load (ml plasma/ml matrix)	Transferrin concentration after depletion (ng/ml)	Achieved transferrin depletion (%)
60	0.3	<30	99.99
600	3.0	200	99.93
800	4.0	200	99.80
1200	6.0	3200	97.20

Table 1. Depletion efficiency. The transferrin values shown after depletion were corrected for an increase in volume due to the dilution from the depletion procedure. The plasma volumes presented in the table are undiluted plasma volumes.

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MATERIALS AND BUFFERS

Sample: Human EDTA plasma diluted 5 times in running buffer.

Column: SulfoLink® Coupling Gel (Pierce cat no 20401) with immobilized Anti-Transferrin Affibody® molecule packed in a Tricorn 5/20 column (GE Healthcare), column volume 0.2 ml.

Chromatography system: ÄKTAexplorer 10S (GE Healthcare)

Running buffer: 2.7 mM KCl, 1.5 mM KH₂PO₄, 320 mM NaCl, 8.1 mM Na₂HPO₄, 0.1% Tween 20, pH 7.4

Elution buffer: 50 mM Glycin-HCl, 100 mM NaCl, 1 M Urea, pH 2.2

EXPERIMENTAL PROCEDURE

For immobilization of Affibody® molecules onto SulfoLink® Coupling Gel from Pierce, see separate protocol on page 3.

The gel was packed in a column which was connected to a chromatography system. Human plasma was diluted five times in running buffer and filtered through a 0.45 µm filter. Different volumes, ranging from 0.3 to 6 ml, of diluted plasma were applied to the column. After each loaded volume of diluted plasma, the column was washed with 30 column volumes of running buffer, followed by elution of bound fraction with 7 column volumes of elution buffer. Absorbance at 280 nm was monitored. The flow rate was 0.2 ml/min throughout the procedure. Flow through and eluted fractions were collected and stored at -20°C.

An ELISA assay was used to analyze the concentration of transferrin in the flow through fraction and in untreated plasma. In the assay, the Anti-Transferrin Affibody® molecule was used as capture reagent and a rabbit anti-transferrin antibody as the detection reagent. The eluted fraction was analyzed at reduced conditions by SDS-PAGE (Invitrogen 12 wells NuPAGE 4-12% Bis-Tris Gel 1.0 mm).

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)

Empty disposable column

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 close the column with the bottom cap whenever the buffer drains down to the top of the bed.
3. Close the column with the bottom cap and add the reduced sample. Retain a small sample of the protein solution for later determination of coupling efficiency.

4. Close the column with 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. Close the column with the bottom cap.
2. Apply 1 ml of blocking buffer.
3. Close the column with 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. Close the column with 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. Close the column with the top cap and store the column upright at 4°C.

LIMITATIONS

Warranty: Affibody[®] products are warranted to meet stated product specifications and to confirm 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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