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Depletion of human serum albumin in embryo culture media for in vitro fertilization using immunoaffinity chromatography

Bottom-up proteomic approach based on tandem mass spectrometry (MS/MS) is a method of choice for identification and quantitation of proteins in the complex biological samples. Followed by quantitative analysis of a protein sample, this approach enables the identification of putative biomarkers for early pathology diagnostics and differentiation. The main challenges confronting this analysis are suppression of low-abundance ions and the limited dynamic range of MS/MS.

Affinity depletion of abundant proteins is an important stage in routine sample preparation prior to tandem mass spectrometry (MS/MS) analysis of biological samples. One such protein is Human Serum Albumin (HSA). In this study, polyclonal αHSA antibodies were immobilised onto a chromatography support to use as immunoaffinity-based column for HSA depletion from embryo culture media for in vitro fertilization (IVF).

HSA depletion from IVF samples using CIMac™ HDZ-αHSA

Diluted IVF culture media was injected onto CIMac™ HDZ-αHSA column in negative mode, where HSA binds on the column, while other proteins remain unbound. The unbound (FT) and elution fractions (EL) were collected according to a length of absorbance peak (350 µL, corresponding to 3-4 column volumes) and processed further according to Scheme 1.

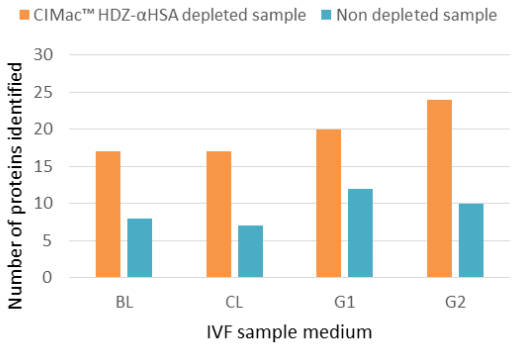
Column	**CIMac™ HDZ-αHSA 0.1 mL Analytical Monolith with 1.3 µm channels**
Sample	IVF culture media diluted in MPA to total protein content of 75–120 µg per injection. Injection volume: 50 µL.
Flow rate	0.3 mL/min
Mobile phases	MPA: PBS, pH 7.4 MPB: Agilent elution buffer, pH 2.25 (Part Number 5185-5988)
Method	Sample injection → MPA wash (5 min), 350 µL FT fraction collection → MPB wash (5 min), 350 µL EL fraction collection
Washing	MPA (4 min) → MPB (3 min) → MPA (13 min)
Regeneration	Not additionally regenerated between analyses.

Above: LC method. Below: Scheme 1: Sample processing workflow.

Fraction collection → Concentration → Denaturation → Alkylation → Trypsin digest → LC-MS/MS → Data analysis

Results

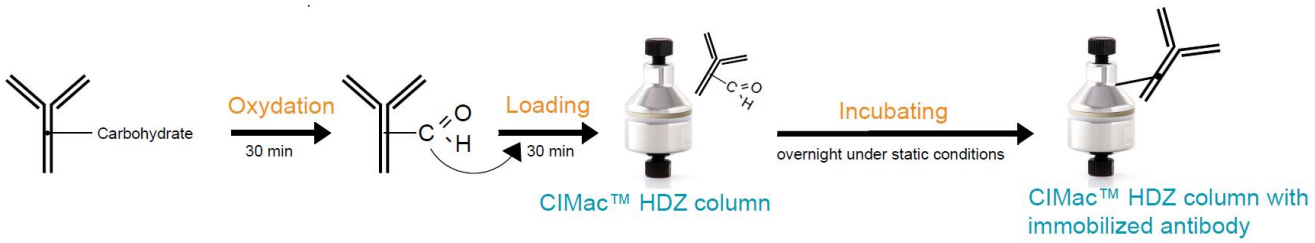
HSA depletion was measured by label-free quantitation from LC-MS analyses. The HSA depletion efficiency determined from FT fractions compared to non-depleted samples was between 70 and 95 %. EL fractions contained 100 % HSA for three out of four IVF samples, proving high specificity for human serum albumin.



Depletion of human serum albumin from the samples nearly doubled the number of identified proteins in all samples (Figure 1).

Figure 1: Number of proteins identified in non-depleted sample and albumin-free fractions from CIMac™ HDZ-αHSA. The number of proteins was averaged over three to six replicates. Only proteins with three or more peptide-spectrum matches were counted

Coupling of polyclonal αHSA antibodies to CIMac™ HDZ (Hydrazide) chromatography column



Hydrazide activation chemistry allows coupling of the antibody through the carbohydrate residues on the Fc region of the molecule, far away from the antibody binding domains. This leaves the binding domains fully accessible to enable the most effective capture of the target compound. Monoliths, due to their large channel structure, eliminate the long loading residence times that are normally required for affinity chromatography. Thus, immobilised monoliths are an excellent choice for high throughput affinity applications.

Polyclonal αHSA antibodies were covalently coupled to CIMac™ HDZ, a 0.1 mL bed volume analytical column, following the manufacturer's recommended procedure. The resulting immunoaffinity CIMac™ HDZ-αHSA column was characterised and the dynamic binding capacity for human HSA measured cca 1.20 mg/mL (Figure 2).

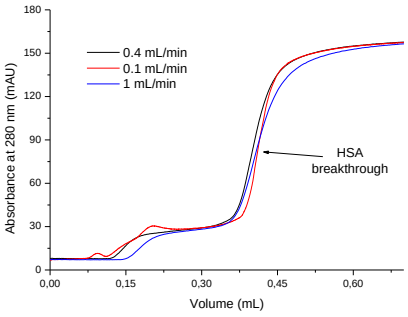


Figure 2: Dynamic binding capacity (DBC) of CIMac™ HDZ-αHSA measured at different flow rates. Analyte: HSA (0.25 mg/mL) in phosphate-buffered saline (PBS). Binding buffer: PBS, pH 7.2. Elution buffer: 0.1 M glycine buffer, pH 2.0. ϕ = 0.4, 0.1, 1 mL/min. λ = 280 nm.

Conclusions

A custom immunoaffinity column was used for HSA depletion from embryo culture media for in vitro fertilization. It was demonstrated that the removal of up to 95% of human albumin resulted in a 50% increase in the number of proteins identified. The high specificity for HSA resulted in no loss of low abundance proteins in most samples tested (3 of 4). These results are promising for the application of CIMac™ HDZ-αHSA monoliths for clinical sample preparation prior to proteomic analysis and fast screening of culture media after embryo incubation.

Materials and methods

Commercially available embryo culture media for in vitro fertilization (IVF) were used as HSA-rich samples: G1/G2 (Vitrolife Sweden AB, Göteborg, Sweden) and Cleavage/Blastocyst (CL/BL) (Cook Medical, Brisbane, Australia). Data was acquired using maXis Impact UHR TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) coupled to a Dionex UltiMate® 3000 RSLC nanoLC system (Dionex-Thermo Scientific, Germering, Germany). Collision-induced dissociation (CID) was used for peptide fragmentation. MS/MS data were acquired in a 300-2000 m/z range using three-second cycle. The proteins were quantified using label-free quantitation approach called normalized spectral index (SIn) as described by Griffin et al., 2010.

Further reading

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- Griffin, N. M., Yu, J., Long, F., Oh, P., Shore, S., Li, Y., Koziol, J. A., Schnitzer, J. E., Nature Biotechnology, 28 (2010) 83–89.

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Product used

Catalogue No.	Product description
110.8002-1.3	CIMac™ HDZ-0.1 Analytical Column (Hydrazide) (Pores 1.3µm)

Similar products

Catalogue No.	Product description
311.8002-2	CIMmultus™ HDZ-1 Advanced Composite Column (Hydrazide) (Pores 2 µm)

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