Enhanced analytical resolution due to efficient depletion of albumin and IgG from human plasma using new prepacked columns

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Background
The most abundant proteins in human plasma are albumin and IgG, which constitute more than 60% of the total protein content. By removing albumin and IgG, the quality and depth of downstream analysis, such as 1-D and 2-D gel electrophoresis and mass spectrometry, can be greatly enhanced.

The new HiTrap™ Albumin & IgG Depletion 1 ml column has been especially developed for handling larger sample volumes of up to 150 µl human plasma. The same media is also available in SpinTrap™ format designed for up to 50 µl human plasma.

The prepacked formats of HiTrap and SpinTrap facilitate fast and convenient sample preparation for different sample volume requirements.

HiTrap Albumin & IgG Depletion
Characteristics
- **Bed volume:** 1 ml
- **Medium:** a mixture of anti-HSA Sepharose™ High Performance and Protein G Sepharose High Performance
- **Loading capacity:** up to 150 µl undiluted human plasma
- **Depletion level:** > 95% HSA and > 90% IgG
- **Recommended flow rate:** 1 ml/min
- **Cycle time:** 35 minutes

Depletion prior to Lectin affinity chromatography
Fractionation of plasma proteins in an number of steps is often required before analytical detection. Depletion of 150 µl human plasma was performed using HiTrap Albumin & IgG Depletion followed by capture of glycosylated proteins by Crotalaria Lectin Sepharose 4 FF in a 1 ml column. For comparison, non-depleted plasma was directly applied to the Lectin column.

Mainly high and medium abundant proteins were identified by LC-MS, while 2-D electrophoresis clearly detected a higher presence of glycosylated IgG (green spots) obtained from non-depleted plasma.

In comparison, depletion of albumin and IgG prior to Lectin affinity chromatography allowed for detection of an additional number of glycosylated proteins in plasma (red spots, see enlargement).

High reproducibility and depletion capacity
HiTrap Albumin & IgG Depletion is designed for depletion of human plasma without dilution of the sample before loading. Six repeated runs using a volume of 150 µl human plasma were performed and the non-bound fractions containing the depleted sample were collected. The ELISA results were >99% albumin depletion and >95% IgG depletion for all six runs, demonstrating high and consistent depletion levels for both albumin and IgG.

**Comparison of depletion efficiencies**
A study comparing HiTrap Albumin & IgG Depletion with the 1 ml column Albumin/IgG Depletion Cartridge (Qiagen®) was performed. The performance during depletion of 150 µl plasma was compared. The depletion run for Albumin/IgG Depletion Cartridge required dilution of the plasma sample and lower flow rate, 0.2 ml/min, during binding (according to the manufacturers description).

ELISA results show that significantly higher albumin and IgG depletion levels were obtained for HiTrap Albumin & IgG Depletion. This was further confirmed by comparison of 2-D PAGE spot maps.

**Effect on analytical resolution**
To evaluate the effect on analytical resolution non-depleted and depleted plasma were analysed by 2-D electrophoresis. Parallel run in the gel and labeling of the samples with different fluorescent dyes made it possible to estimate the differences in protein abundances.

A comparison of protein spot maps for non-depleted plasma and albumin/IgG-depleted plasma show enhanced visualization of proteins with isoelectric points and/or molecular weight similar to albumin and IgG (marked with circles). In addition, an increased number of less abundant proteins were detected (green spots).

**Conclusions**
HiTrap Albumin & IgG Depletion provides the following benefits:
- Efficient sample preparation tool for fractionation of plasma proteins
- High depletion levels for large sample volumes, up to 150 µl undiluted plasma
- Fast and easy depletion procedure
- High reproducibility for repeated runs on the same column
- Convenient prepacked format easily used with chromatography systems such as ÄKTA™ design