# **HiTrap** Albumin and IgG Depletion Albumin and IgG Depletion **SpinTrap**

PROTEIN SAMPLE PREPARATION

HiTrap™ Albumin and IgG Depletion and Albumin and IgG Depletion SpinTrap™ are prepacked columns for the depletion of albumin and IgG from human serum and plasma (Fig 1). The columns are prepacked with high performance Sepharose™ based media with an affinity for human serum albumin (HSA) and IgG. These columns are members of the Trap platform and address the need for flexible, small-scale preparation of protein samples prior to downstream analyses such as 1-D or 2-D gel electrophoresis and mass spectrometry.

A common problem when studying plasma or serum is the difficulty in detecting less abundant proteins. The most abundant plasma proteins are albumin and IgG, and they tend to obscure the signals of less abundant proteins. The high abundance of albumin and IgG also interferes with the detection of other proteins by preventing a sufficient amount of less abundant proteins from being included in the analysis. By depleting samples of albumin and IgG, the quality and depth of the analysis can be greatly enhanced. Depletion of the two proteins removes more than 60% of the total protein content in human plasma, allowing proteins normally obscured by albumin and IgG to be visualized.

Key benefits:

- High depletion capacity, removes > 95% albumin and > 90% IgG, with high reproducibility
- Removal of albumin and IgG allows a higher load of less abundant proteins to be included in the analysis and detection of more proteins
- Simple and fast procedure
- HiTrap Albumin and IgG Depletion provides rapid and easy processing in a convenient format for handling larger sample volumes (~150 µl) using a liquid chromatography system or manually using a syringe
- Albumin and IgG Depletion SpinTrap handles smaller sample volumes (~50 µl) using a tabletop centrifuge

**Fig 1.** HiTrap Albumin and IgG Depletion and Albumin and IgG Depletion SpinTrap for simple and rapid depletion of albumin and IgG from human serum and plasma samples.

## Description

Both columns are prepacked with a mixture of anti-HSA Sepharose High Performance and Protein G Sepharose High Performance. The mixed chromatography medium consists of highly cross-linked agarose beads with covalently immobilized affinity ligands. The ligand for anti-HSA Sepharose High Performance is based on a single domain antibody fragment with high specificity and capacity for HSA. The ligand for Protein G Sepharose High Performance is derived from the IgG binding regions of Protein G, a cell surface protein of *Streptococcus* bacteria. The Protein G ligand binds human IgG1, IgG2, IgG3, and IgG4. It is also effective in removing IgG from rat and mouse plasma.

### *HiTrap Albumin and IgG Depletion*

HiTrap Albumin and IgG Depletion 1 ml column is designed for the depletion of albumin and IgG from larger sample volumes (~150 µl) of human plasma or serum. The column characteristics are summarized in Table 1. HiTrap columns are made of biocompatible polypropylene that does not interact with biomolecules. Note that HiTrap columns cannot be opened or refilled.

HiTrap Albumin and IgG Depletion is designed for a sample volume of approximately 150 µl human plasma or serum. Application of 150 µl undiluted sample, containing normal levels of albumin and IgG (~40 mg HSA/ml and ~15 mg IgG/ml), results in > 95% albumin depletion and > 90% IgG depletion. When applying sample containing albumin and IgG above normal levels, it is recommended to use a lower sample volume (100 µl to 125 µl) to obtain the same depletion efficiency.

The depletion procedure is simple and rapid (Fig 2), taking approximately 35 min to complete, and no dilution of human plasma is required. Apply filtered but unclarified sample, and process using a liquid chromatography system such as an ÄKTA™ system, a peristaltic pump, or operate manually using a syringe.

**Table 1.** HiTrap Albumin and IgG Depletion characteristics

| | |
|---|---|
| Matrix | Highly cross-linked 6% agarose |
| Average particle size | 34 µm |
| Ligands | Recombinant Protein G fragment and recombinant protein binding HSA |
| Loading capacity[1] | 150 µl undiluted human plasma |
| Column volume | 1 ml |
| Column dimensions, i.d. × H | 0.7 × 2.5 cm |
| Recommended flow rate | 1 ml/min |
| Maximum flow rate | 4 ml/min |
| Column hardware pressure limit | 5 bar (0.5 MPa, 70 psi) |
| pH stability | |
| Cleaning[2] | 2 to 9 |
| Working[3] | 3 to 9 |
| Storage | 20% ethanol at 4°C to 8°C |

[1] Sample: Human plasma containing ~40 mg albumin/ml and ~15 mg IgG/ml. Results according to ELISA: > 95% albumin depletion and > 90% IgG depletion.

[2] Refers to the pH interval for regeneration.

[3] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

### *Albumin and IgG Depletion SpinTrap*

Albumin and IgG Depletion SpinTrap is designed for smaller volumes (~50 µl) of human plasma or serum. The column characteristics are summarized in Table 2. Application of 50 µl undiluted human plasma, containing normal levels of albumin and IgG (~40 mg HSA/ml and ~15 mg IgG/ml), results in > 95% albumin depletion and > 90% IgG depletion. For plasma containing levels of albumin and IgG above the normal range, it is recommended to apply a lower sample volume, for example 25 µl, to obtain the same depletion efficiency.

The depletion of plasma or serum with Albumin and IgG Depletion SpinTrap can be divided into three stages: equilibration, sample application, and collection of the depleted fraction (Fig 2). The protocol requires a tabletop centrifuge and can be completed in only 10 min. Before application to the column, the plasma sample is diluted with binding buffer to a final volume of 100 µl.

**Table 2.** Albumin and IgG Depletion SpinTrap characteristics

| | |
|---|---|
| Matrix | Highly cross-linked 6% agarose |
| Average particle size | 34 µm |
| Ligands | Recombinant Protein G fragment and recombinant protein binding HSA |
| Loading capacity[1] | 50 µl undiluted human plasma |
| Volume, prepacked medium | 370 µl (600 µl medium slurry) |
| Column material | Polypropylene barrel and polyethylene frits |
| Working pH range[2] | 2 to 9 |
| Storage | 20% ethanol at 4°C to 8°C |

[1] Sample: Human plasma containing ~40 mg albumin/ml and ~15 mg IgG/ml. Results according to ELISA: > 95% albumin depletion and > 90% IgG depletion.

[2] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance

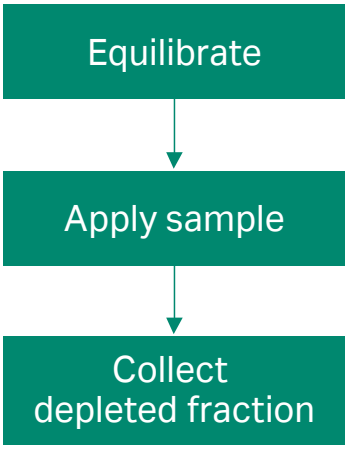



**Fig 2.** The principle for the depletion procedure using HiTrap Albumin and IgG Depletion and Albumin and IgG Depletion SpinTrap is rapid and convenient, completed in only a few simple steps. Note that, at the end of the depletion procedure for HiTrap Albumin and IgG Depletion, albumin and IgG are eluted and the column is re-equilibrated before the next depletion run.

### *High reproducibility and depletion capacity*

#### HiTrap Albumin and IgG Depletion

The same HiTrap Albumin and IgG Depletion column may be used for a limited number of repeated runs, which is cost effective and convenient. In the experiment below, six repeated runs using a sample volume of 150 µl undiluted plasma were performed (Fig 3). The column was re-equilibrated with binding buffer between the runs. Figure 3 shows an overlay of the chromatograms from the six runs, illustrating very high reproducibility. ELISA results demonstrate both high and consistent depletion rates, 99% to 100% for albumin and 95% to 96% for IgG (data not shown).

| | |
|---|---|
| *Column:* | HiTrap Albumin and IgG Depletion, 1 ml |
| *Sample:* | Human plasma |
| *Sample volume:* | 150 µl |
| *Binding buffer:* | 20 mM sodium phosphate, 150 mM NaCl, pH 7.4 |
| *Elution buffer:* | 0.1 M glycine-HCl, pH 2.7 |
| *Flow rate:* | 1 ml/min |
| *System:* | ÄKTAexplorer |

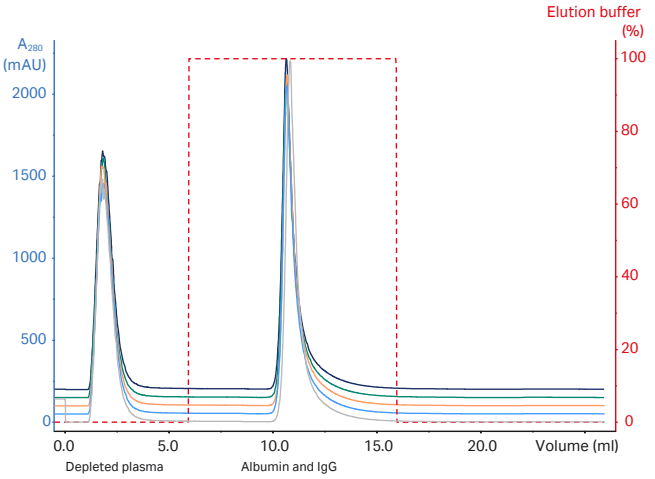



**Fig 3.** Six repeated runs using the same HiTrap Albumin and IgG Depletion column shows high reproducibility.

#### Albumin and IgG Depletion SpinTrap

High reproducibility is very important for parallel sample preparation. Replicate depletion runs were performed on ten Albumin and IgG Depletion SpinTrap columns using a load corresponding to 50 µl plasma. Figure 4 shows ELISA results above 99% depletion for both albumin and IgG, demonstrating consistent column performance and high reproducibility.

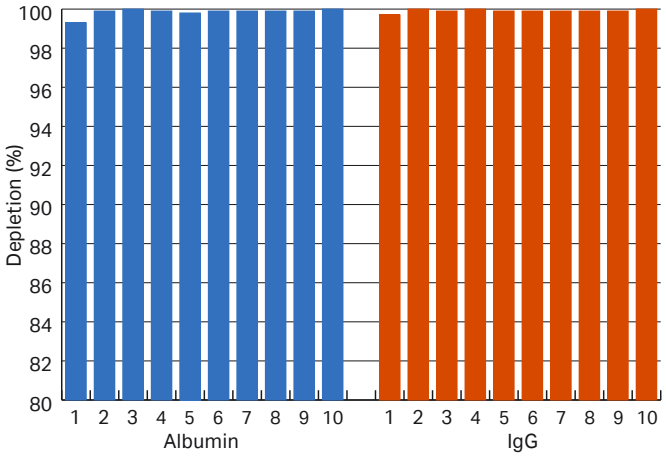



**Fig 4.** Ten parallel runs using Albumin and IgG Depletion SpinTrap columns demonstrate very high reproducibility. Note that the y-axis ranges from 80% to 100%.

## Applications

### *1. Depletion of undiluted human plasma*

HiTrap Albumin and IgG Depletion can be used for depletion of human plasma without dilution of the sample before loading. A volume of 150 µl human plasma was applied to the column, and the non-bound fraction containing the depleted sample was collected. The depletion of albumin and IgG is shown by SDS-PAGE analysis (Fig 5). The depletion level was also determined by ELISA, and the result for the non-bound fraction was 99% albumin depletion and 98% IgG depletion.

**Lane**
1. Low molecular weight marker
2. Human plasma
3. Depleted fraction
4. Bound fraction containing albumin and IgG

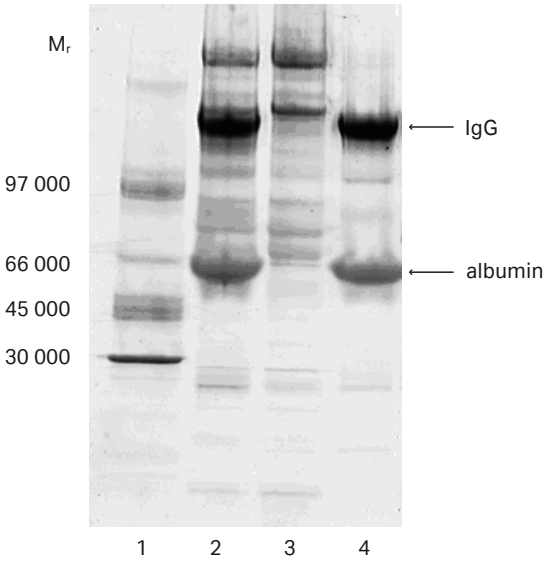



**Fig 5.** Deep Purple stained SDS-PAGE analysis (non-reduced conditions) of fractions from the depletion of human plasma using HiTrap Albumin and IgG Depletion.

To evaluate the effects on analytical resolution, non-depleted and depleted plasma samples were analyzed by 2-D electrophoresis. 2-D protein spot maps show enhanced visualization, in depleted samples, of proteins with isoelectric points and/or molecular weights similar to albumin and IgG (see circles, Fig 6). In addition, the depletion allowed for a relatively higher load of less abundant proteins, enabling detection of a larger number of proteins and stronger signals (see arrows for examples, Fig 6).

(A) (B)

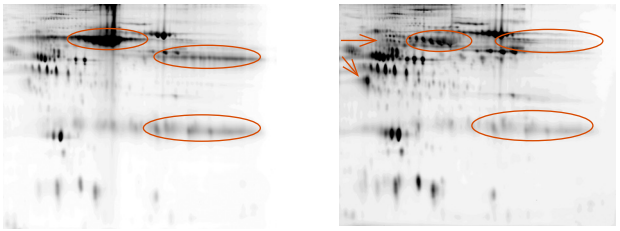


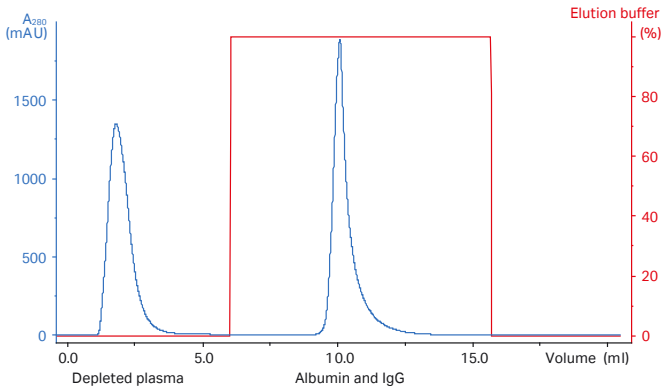
**Fig 6.** 2-D spot maps using a (A) non-depleted plasma sample or (B) a sample depleted using HiTrap Albumin and IgG Depletion column. Performing 2-D electrophoresis on albumin- and IgG-depleted samples gives high resolution protein spot maps, enabling detection of a larger number of proteins.

## *2. Comparison study*

A study comparing HiTrap Albumin and IgG Depletion with the 1 ml column Albumin/IgG Depletion Cartridge (Qiagen™, USA) was performed. The depletion runs were performed at Cytiva laboratories according to the manufacturers' instructions. The depletion of albumin and IgG in the collected non-bound fractions (Fig 7) was determined by ELISA (Fig 8). Figure 8 shows that the degree of depletion was substantially higher for HiTrap Albumin and IgG Depletion compared to the results obtained using the Albumin/IgG Depletion cartridge. In addition, the depletion procedure for HiTrap Albumin and IgG Depletion is shorter, since nodilution of the sample is required, and because of the 1 ml/min flow rate maintained during sample application.

(A)

| | |
|---|---|
| *Column* | HiTrap Albumin and IgG Depletion, 1 ml |
| *Sample:* | Human plasma |
| *Sample volume:* | 150 µl |
| *Binding buffer:* | 20 mM sodium phosphate, 150 mM NaCl, pH 7.4 |
| *Elution buffer:* | 0.1 M glycine-HCl, pH 2.7 |
| *Flow rate:* | 1 ml/min |
| *System:* | ÄKTAexplorer |




(B)

| | |
|---|---|
| *Column:* | Albumin/IgG Depletion Cartridge (Qiagen) |
| *Sample:* | Human plasma |
| *Sample volume:* | 150 µl diluted to 600 µl with binding buffer |
| *Binding buffer:* | 50 mM sodium phosphate, 150 mM NaCl, pH 7.2 |
| *Elution buffer:* | 20 mM glycine-HCl, pH 2.0 |
| *Flow rate:* | 1 ml/min (0.1 ml/min during binding) |
| *System:* | ÄKTAexplorer |

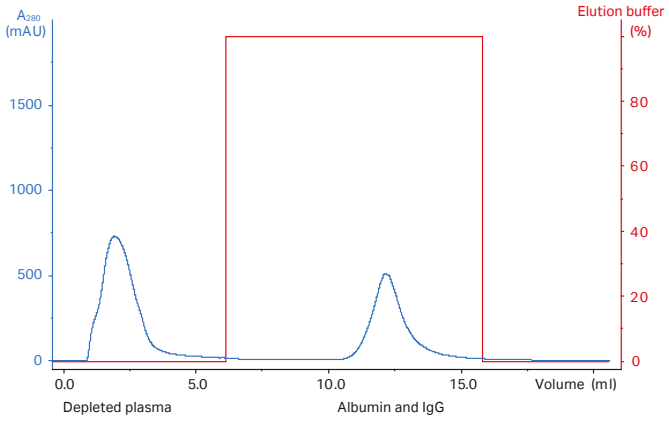



**Fig 7.** Depletion of albumin and IgG using (A) HiTrap Albumin and IgG Depletion and (B) Albumin/IgG Depletion Cartridge from Qiagen. Differences in running conditions are due to the different recommended conditions given by the manufacturers.

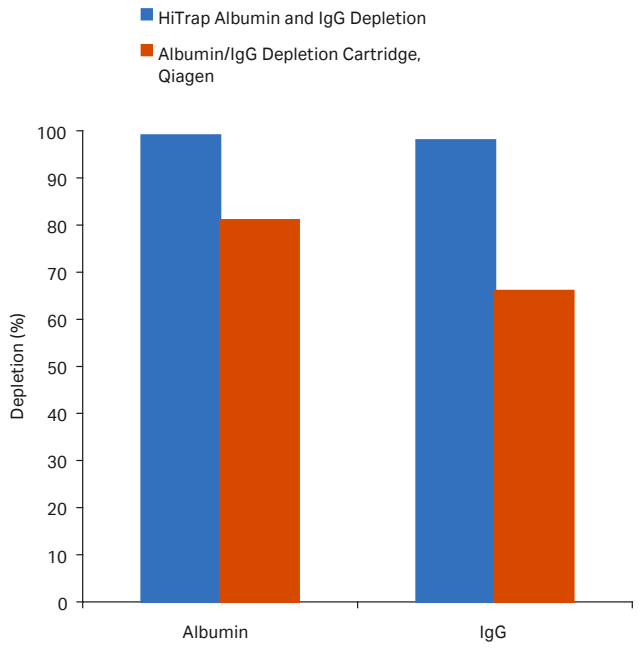



**Fig 8.** Comparison study shows that HiTrap Albumin and IgG Depletion column gives a higher depletion level of albumin and IgG compared to Albumin/IgG Depletion Cartridge from Qiagen.

Samples depleted using either HiTrap Albumin and IgG Depletion or Albumin/IgG Depletion Cartridge were analyzed by 2-D electrophoresis. The 2-D protein spot maps from the analysis show undepleted albumin and IgG in the samples (Fig 9). HiTrap Albumin and IgG Depletion column gave a higher depletion rate, shown by ELISA, and this can also be visualized on the 2-D spot maps.

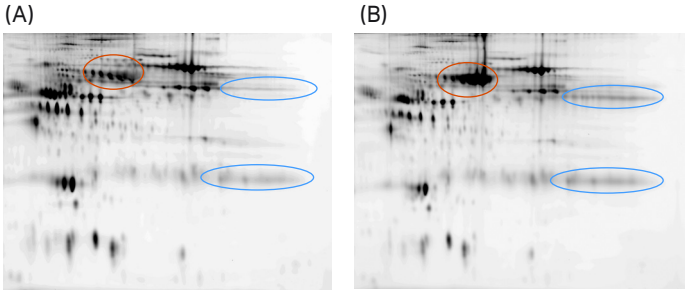



**Fig 9.** 2-D spot maps show remaining albumin (orange circles) and IgG (blue circles) in samples depleted using (A) HiTrap Albumin and IgG Depletion column or (B) Albumin/IgG Depletion Cartridge (Qiagen).

# Ordering information

| Product | Quantity | Code Number |
|---|---|---|
| HiTrap Albumin and IgG Depletion | 2 × 1 ml | 28-9466-03 |
| Albumin and IgG Depletion SpinTrap | 10 | 28-9480-20 |

| Related products | Quantity | Code Number |
|---|---|---|
| 2-D Quant Kit | 500 assays | 80-6483-56 |
| Nuclease Mix | 0.5 ml | 80-6501-42 |
| Protease Mix | 1 ml | 80-6501-23 |
| 2-D Clean-Up Kit | 50 samples | 80-6484-51 |
| SDS-PAGE Clean-Up Kit | 50 samples | 80-6484-70 |
| Vivaspin™ 500 MWCO 3000[1] | 25 samples | 28-9322-18 |
| Vivaspin 500 MWCO 5000 | 25 samples | 28-9322-23 |
| Vivaspin 500 MWCO 10 000 | 25 samples | 28-9322-25 |
| Vivaspin 500 MWCO 30 000 | 25 samples | 28-9322-35 |
| Vivaspin 500 MWCO 50 000 | 25 samples | 28-9322-36 |
| Vivaspin 500 MWCO 100 000 | 25 samples | 28-9322-37 |

[1] Additional Vivaspin formats are available, please see www.cytiva.com/sampleprep.

| Accessories | Quantity | Code Number |
|---|---|---|
| 1/16" male/luer female* | 2 | 18-1112-51 |
| Tubing connector flangeless/M6 female | 2 | 18-1003-68 |
| Tubing connector flangeless/M6 male | 2 | 18-1017-98 |
| Union 1/16" female/M6 male | 6 | 18-1112-57 |
| Union M6 female /1/16" male | 5 | 18-3858-01 |
| Union luerlock female/M6 female | 2 | 18-1027-12 |
| HiTrap/HiPrep™, 1/16" male connector for ÄKTA design | 8 | 28-4010-81 |
| Stop plug female, 1/16"† | 5 | 11-0004-64 |
| Fingertight stop plug, 1/16"‡ | 5 | 11-0003-55 |

* One connector included in each HiTrap package.
† Two, five, or seven stop plugs female included in HiTrap packages depending on products.
‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

| Related Literature | Code Number |
|---|---|
| 2-D Electrophoresis Handbook, Principles and Methods | 80-6429-60 |
| Ettan™ DIGE System User Manual | 18-1173-17 |
| Recombinant Protein Purification Handbook, Principles and Methods | 18-1142-75 |
| Affinity Chromatography Handbook, Principles and Methods | 18-1022-29 |
| Affinity Chromatography Columns and Media, Selection guide | 18-1121-86 |
| HiTrap Column guide | 18-1129-81 |

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