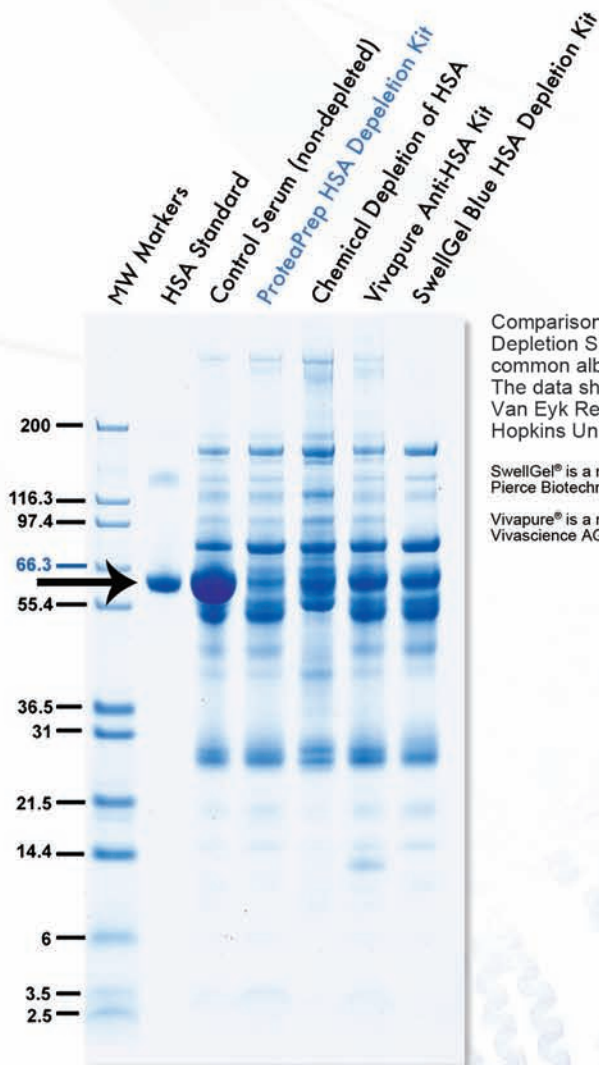




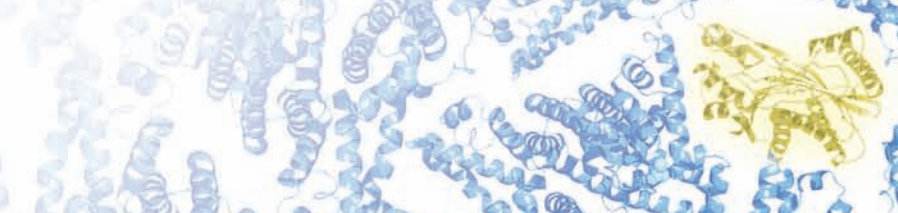
ProteaPrep™ Albumin and IgG Depletion & Capture Sample Prep Kits



Comparison of ProteaPrep Albumin Depletion Sample Prep Kit to other common albumin depletion methods. The data shown is courtesy of the Van Eyk Research Group at Johns Hopkins University, Baltimore, MD.

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ProteaPrep™ Albumin and IgG Depletion and Capture Sample Prep Kits

Albumin and IgG are two of the most highly abundant proteins found in serum, and are often the topic of conversation when it comes to serum proteomics. These proteins have important functions and are vital to many biological processes, but can often times mask other, lower abundant proteins of interest. The selective removal of albumin and IgG from samples provides increased visibility to the lower abundant proteins. After the removal of these highly abundant proteins, researchers are then able to evaluate other lower abundant proteins and biomarkers in serum.

Using a new ligand design, we've created a method for depleting and capturing both albumin and IgG from serum samples with very high efficiency. The ProteaPrep Depletion and Capture Sample Prep Kits can quickly remove >99% of albumin and/or IgG from serum samples. The high efficiency is possible due to the capture ligand – a recombinant protein. More specific than an antibody-based system, the recombinant protein also produces binding constants to both human serum albumin and IgG that are much stronger than traditional antibody systems.

All of the ProteaPrep Depletion and Capture kits are formulated in a convenient, disposable SpinTube design and have a protocol that is very quick and easy to complete. Multiple options are available for independent or combined albumin and IgG depletion and capture. All kits function effectively with a variety of different organisms. Your protein bands and spots will no longer be masked by the overabundance of albumin or IgG in the samples!

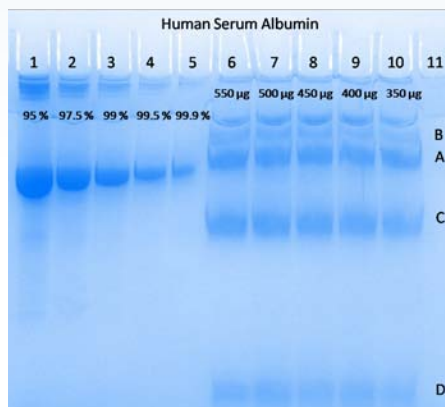


ProteaPrep™ Albumin Depletion Sample Prep Kit

Protea now offers a way to virtually eliminate albumin from your serum samples! The ProteaPrep Albumin Depletion Sample Prep Kit quickly removes >99% of albumin from serum samples. This technology produces binding constants with human serum albumin that are significantly stronger than antibody-based methods.

Features and Benefits

- >99% efficiency for depletion of human serum albumin
- Simple and easy to use – process samples in less than 20 minutes
- Albumin binding affinity 2 to 3 orders of magnitude greater than traditional antibody-based albumin depletion methods



Depletion of human serum albumin (HSA) from standard samples (350 - 550 µg per sample, lanes 6 to 10). Lanes 1 to 5 show control loads of HSA for determination of depletion efficiency. >99% of HSA is removed from the sample. At the high protein sample loads shown, low abundance contaminant proteins (<0.1 to 1% of sample) are enriched to detectable limits in the coomassie blue stained gel separation. Key: A) HSA B) plasma protease C1 inhibitor C) haptoglobin D) transthyretin.

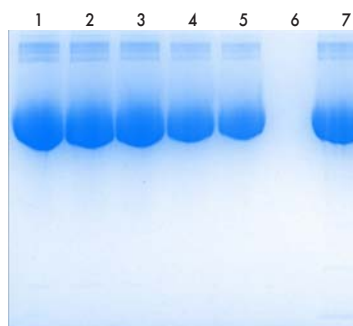
Catalog Number	Description	Number of Preps	Price
SP-200-12	ProteaPrep Albumin Depletion Sample Prep Kit, Contains 12 SpinTubes with functionalized beads and 25mL Bead Activation Buffer	12 Prep	\$385
SP-200-24	ProteaPrep Albumin Depletion Sample Prep Kit, Contains 24 SpinTubes with functionalized beads and 25mL Bead Activation Buffer	24 Prep	\$770
SP-200-4x24	ProteaPrep Albumin Depletion Sample Prep Kit, Contains 96 SpinTubes with functionalized beads and 4 x 25mL Bead Activation Buffer	96 Prep	\$3080

ProteaPrep™ Albumin Capture Sample Prep Kit

The ProteaPrep Albumin Capture Sample Prep Kit (SP-210) incorporates a reversible binding recombinant affinity ligand for highly efficient capture (>99%) and release (>80%) of human serum albumin from plasma and serum samples in less than 30 minutes.

Features and Benefits

- >99% efficiency for depletion of human serum albumin
- >80% efficiency for recovery of human serum albumin



Capture and recovery of 400 µg human serum albumin (HSA) using the SP-210 ProteaPrep Albumin Capture Sample Prep Kit. Lanes 1-5 show recovery controls based on 90%, 80%, 70%, 60%, 50% of 400 µg of HSA, respectively. Lane 6 is blank. Lane 7 shows the eluate from 400 µg of HSA captured and recovered with 80% efficiency using the SP-210 ProteaPrep Albumin Capture Sample Prep Kit.

Catalog Number	Description	Number of Preps	Price
SP-210-12	ProteaPrep Albumin Capture Sample Prep Kit Contains 12 SpinTubes with functionalized beads, 25 mL Bead Activation Buffer, 25 mL Albumin Release Reagent	12 Prep	\$425
SP-210-24	ProteaPrep Albumin Capture Sample Prep Kit Contains 24 SpinTubes with functionalized beads, 25 mL Bead Activation Buffer, 25 mL Albumin Release Reagent	24 Prep	\$850
SP-210-4x24	ProteaPrep Albumin Capture Sample Prep Kit Contains 4 X 24 SpinTubes with functionalized beads, 25 mL Bead Activation Buffer, 4 x 25 mL Albumin Release Reagent	96 Prep	\$3400

ProteaPrep™ Albuminome Analysis Sample Prep Kit

The ProteaPrep Albuminome Analysis Sample Prep Kit (SP-205) features the ProteaPrep Albumin Depletion Sample Prep Kit (SP-200) for efficient capture of the HSA on the recombinant protein ligand and a Trypsin Digestion Kit (PE-151) for on-bead digestion of the human serum albumin and any proteins that are bound or associated with the HSA.

Parameters	On-bead Digestions of Albuminome Analyzed by LC-MS/MS						
	25	60	60	60	90	90	90
Gradient Time (min)	25	60	60	60	90	90	90
Volume Injected (μL)	15	15	30	45	15	30	45
% Total Albuminome Injected	4.2%	4.2%	8.4%	12.6%	4.2%	8.4%	12.6%
Total Proteins ID >95%	36	53	61	58	63	76	85

Table 1: On Bead Digestions of Albuminome. 10μL aliquots of human serum were processed using ProteaPrep Albuminome Sample Prep Kit SpinTubes. After removal of the filtrate (non-adsorbed components), the functionalized beads were removed from the SpinTube and proteolyzed with trypsin to digest the albumin and the associated bound protein fraction. Aliquots of a single digest were analyzed by LC-MS/MS (Thermo LTQ-XL mass spectrometer) under different experimental conditions (e.g. gradient time and injection volume) to identify the components of the albuminome. Data analysis was performed with ProteinPilot™ software.

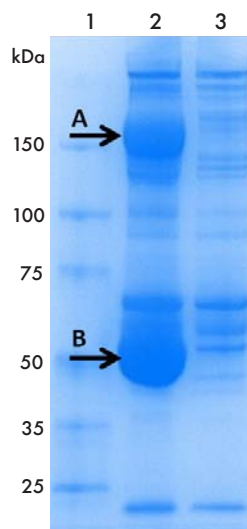
Catalog Number	Description	Number of Preps	Price
SP-205-12	ProteaPrep Albuminome Analysis Sample Prep Kit Contains 12 SpinTubes with functionalized beads , 25 mL Bead Activation Buffer, Trypsin Digestion Kit	12 Prep	\$495
SP-205-24	ProteaPrep Albuminome Analysis Sample Prep Kit Contains 24 SpinTubes with functionalized beads , 25 mL Bead Activation Buffer, Trypsin Digestion Kit	24 Prep	\$990
SP-205-4x24	ProteaPrep Albuminome Analysis Sample Prep Kit Contains 4 X 24 SpinTubes with functionalized beads , 4x25 mL Bead Activation Buffer, Trypsin Digestion Kit	96 Prep	\$3960

ProteaPrep™ Albumin & IgG Depletion Sample Prep Kit

The ProteaPrep Albumin and IgG Depletion Sample Prep Kit (SP-240) selectively removes both albumin and IgG. The functionalized beads irreversibly bind and remove the albumin and IgG from serum in a highly efficient and rapid manner. Depletion efficiencies are >99% for the selective removal of both albumin and IgG in a single SpinTube from serum samples in less than 20 minutes.

Features and Benefits

- >99% efficiency for depletion of both human serum albumin and IgG
- Simple and easy to use - process samples in less than 20 minutes
- Effectively remove albumin and IgG in one step



>99% Depletion of human serum albumin (HSA) and IgG from 10 μL of human serum using the SP-240 ProteaPrep Albumin and IgG Depletion Sample Prep Kit. The upper (A) and lower (B) black arrows indicate the HSA and IgG protein bands, respectively, in the control sample that are not observed in the depleted sample. Key: Lane 1 = molecular weight markers ; Lane 2 = 10 μL human serum control, 10 % sample load ; Lane 3 = 10 μL human serum, HSA and IgG depleted, 10 % sample load.

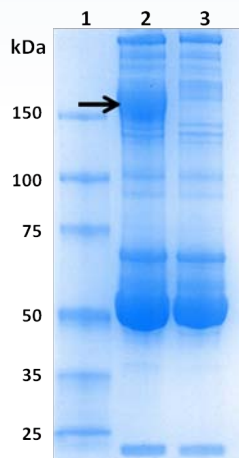
Catalog Number	Description	Number of Preps	Price
SP-240-12	ProteaPrep Albumin and IgG Depletion Sample Prep Kit Contains 12 SpinTubes with functionalized beads, 25 mL Bead Activation Buffer	12 Prep	\$575
SP-240-24	ProteaPrep Albumin and IgG Depletion Sample Prep Kit Contains 24 SpinTubes with functionalized beads, 25 mL Bead Activation Buffer	24 Prep	\$1150
SP-240-4x24	ProteaPrep Albumin and IgG Depletion Sample Prep Kit Contains 4 X 24 SpinTubes with functionalized beads, 4x25 mL Bead Activation Buffer	96 Prep	\$4600

ProteaPrep™ IgG Depletion Sample Prep Kit

The ProteaPrep IgG Depletion Sample Prep Kit (SP-220) selectively removes IgG. The ProteaPrep IgG Depletion capture ligand is a recombinant protein that binds with high affinity to IgG and is highly purified for efficient removal of IgG from human serum samples. Depletion efficiencies are >99% for the selective removal of IgG from serum samples in less than 20 minutes.

Features and Benefits

- >99% efficiency for depletion of human IgG
- Simple and easy to use – process samples in less than 20 minutes
- Disposable, cost-effective SpinTube design eliminates the risk of carryover or sample contamination



>99% Depletion of IgG from 10 µL of human serum using the SP-220 ProteaPrep IgG Depletion Sample Prep Kit. The black arrow indicates the IgG protein band in the control sample that is not observed in the depleted sample.

Key: Lane 1 = molecular weight marker; Lane 2 = 10 µL human serum control, 10 % sample load; Lane 3 = 10 µL human serum, IgG depleted, 10 % sample load.

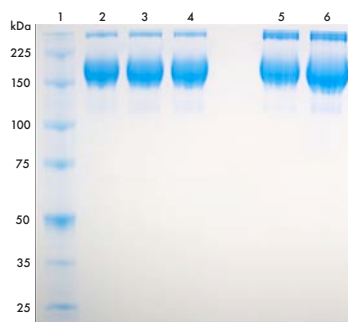
Catalog Number	Description	Number of Preps	Price
SP-220-12	ProteaPrep IgG Depletion Sample Prep Kit Contains 12 SpinTubes with functionalized beads, 25 mL Bead Activation Buffer	12 Prep	\$385
SP-220-24	ProteaPrep IgG Depletion Sample Prep Kit Contains 24 SpinTubes with functionalized beads, 25 mL Bead Activation Buffer	24 Prep	\$770
SP-220-4x24	ProteaPrep IgG Depletion Sample Prep Kit Contains 4 X 24 SpinTubes with functionalized beads, 4x25 mL Bead Activation Buffer	96 Prep	\$3080

ProteaPrep™ IgG Capture Sample Prep Kit

The ProteaPrep IgG Capture Sample Prep Kit (SP-230) incorporates a reversible binding recombinant affinity ligand for highly efficient capture (>99%) and release (>80%) of IgG from plasma and serum samples in less than 30 minutes.

Features and Benefits

- >99% efficiency for depletion of human IgG
- >80% efficiency for recovery of human IgG



Capture and recovery of 250 µg human immunoglobulin G (IgG) using the SP-230 ProteaPrep IgG Capture Sample Prep Kit. Lane 1 contains a molecular weight marker. Lanes 2 to 4 show recovery controls based on 70%, 80%, and 90% of 250 µg of IgG, respectively. Lanes 5 and 6 shows the eluate from 250 µg of IgG from two different vendors that was captured and recovered with >80% efficiency using the SP-230 ProteaPrep IgG Capture Sample Prep Kit. The gel is a 7.5% ProteaGel™ stained with Coomassie Blue G-250 for visualization of the proteins.

Catalog Number	Description	Number of Preps	Price
SP-230-12	ProteaPrep IgG Capture Sample Prep Kit Contains 12 SpinTubes with functionalized beads , 25 mL Bead Activation Buffer, 25 mL IgG Release Reagent	12 Prep	\$425
SP-230-24	ProteaPrep IgG Capture Sample Prep Kit Contains 24 SpinTubes with functionalized beads , 25 mL Bead Activation Buffer, 25 mL IgG Release Reagent	24 Prep	\$850
SP-230-4x24	ProteaPrep IgG Capture Sample Prep Kit Contains 4 X 24 SpinTubes with functionalized beads , 4x25 mL Bead Activation Buffer, 4 x 25 mL IgG Release Reagent	96 Prep	\$3400

Introduction

One of the major problems faced in the analysis of the human serum proteome is the broad dynamic range of its protein constituents. High abundance proteins, such as human serum albumin (HSA) and gamma-immunoglobulin (IgG), which together comprise 75% of total serum protein content, inhibit the analysis of lower abundance proteins of interest. Current research is centered on performing proteomics analysis of serum/plasma for the characterization of proteins in human serum, as well as the discovery of new biomarkers.

Affinity-based depletion strategies are often employed to selectively remove high abundance proteins, such as HSA and IgG, in order to enrich the lower abundance proteins and facilitate their analysis. Traditionally, antibody-based schemes are used to produce affinity-binding ligands for HSA and IgG. Variability in the production of these antibodies leads to a wide variety of specificities and selectivities, as the binding of the ligand to the HSA or IgG is dependent upon the availability of the epitope, the binding affinity of the antibody, and the homogeneity of the antibody population. Thus, the efficiencies for antibody-based depletion strategies for the selective removal of high abundance proteins in human serum can vary broadly, from 70 to 95%, and can dramatically affect the reproducibility of studies of the human serum proteome.

The ProteaPrep Albumin Depletion Sample Prep Kit (SP-200) features a revolutionary new approach to affinity-based serum albumin removal that utilizes a non-antibody-based method. The ProteaPrep albumin depletion capture ligand is a recombinant protein that exhibits excellent batch-to-batch reproducibility and is highly purified for robust, efficient removal of albumin from human serum samples. This proprietary ligand design technology produces binding constants with human serum albumin ($K_d \sim 1E-11M$) that are significantly stronger than antibody-based methods, which have binding constants that range from K_d 's of $1E-6$ to $1E-8M$. The net result is that the functionalized beads irreversibly bind and remove albumin from serum in a highly efficient and rapid manner.

Depletion efficiencies are >99% for the selective removal of HSA from serum samples in less than 20 minutes. The ProteaPrep Albumin Depletion Sample Prep Kit has also been shown to be effective for the depletion of albumin from the sera of other species.

Experimental

To evaluate the efficiency of the ProteaPrep Albumin Depletion sample prep kit (SP-200), initial experiments were conducted on standard samples of HSA as well as human serum samples. Samples of standard HSA (350 – 550 μg HSA) and human serum (1 – 10 μL) were processed with the ProteaPrep albumin depletion kit. Depleted samples were analyzed by 1D SDS-PAGE gel (10% ProteaGel, PG-402D) and visualized by Coomassie staining (SB-G250). Western Blot analysis was also conducted to further demonstrate the effectiveness of the ProteaPrep albumin depletion SpinTubes, as well as to confirm the removal of HSA from human serum. Depleted samples were run on a 1D SDS-PAGE gel (10% ProteaGel) followed by Western Blot probing with an anti-HSA antibody.

The total time for the SpinTube procedure for albumin depletion takes less than 20 minutes. Prior to analysis by SDS-PAGE, 2D gel, or mass spectrometry, it may be necessary to desalt the sample. We recommend using a centrifugal ultrafiltration unit (SP-021 or SP-022) to remove serum salts, buffer salts, and small molecular weight species from the sample prior to downstream applications. The sample prep workflow is illustrated in Figure 1.

To demonstrate the utility of the ProteaPrep Albumin Depletion sample prep kit, depletion efficiencies were evaluated for serum from several species of interest.

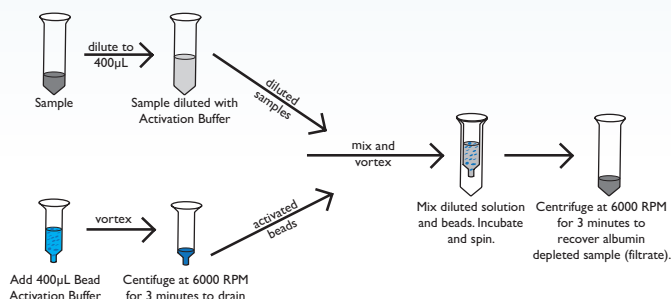


Figure 1. Workflow for albumin depletion

Results and Discussion

The effectiveness of the ProteaPrep Albumin Depletion Sample Prep Kit for the removal of HSA from human serum albumin was evaluated. 1 μL from two, unique lots of human serum was depleted using the kits. Depleted samples plus control samples of 1 μL of the matched, non-depleted human serum were analyzed by 1D SDS-PAGE gel and visualized by Coomassie staining to determine depletion efficiency (Figure 2).

The black arrow in Figure 2 indicates the HSA protein band in the human serum samples (Lanes 2 and 4). In the depleted samples (Lanes 3 and 5), the HSA band is not observed, indicating >99% HSA depletion.

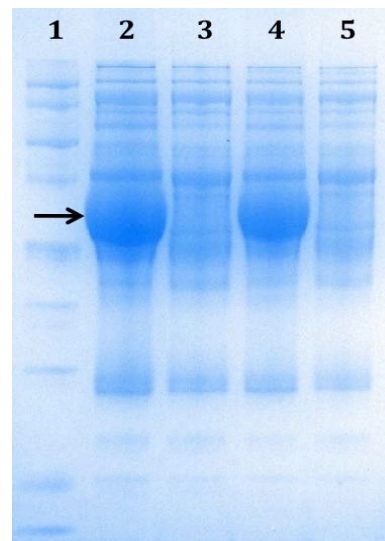


Figure 2. Coomassie blue stained SDS-PAGE gel demonstrating the efficiency of the SP-200 ProteaPrep Albumin Depletion Sample Prep kit for the removal of HSA from human serum samples. Key: Lane 1 = molecular weight markers ; Lanes 2 and 4 = 1.0 μL of two unique human serum control samples with the prominent HSA bands marked with an arrow ; Lanes 3 and 5 = albumin-depleted 1.0 μL samples.

Western Blot analysis of HSA depleted samples was also conducted to further demonstrate the effectiveness of the ProteaPrep Albumin Depletion Sample Prep Kit and to confirm the removal of HSA. It was demonstrated that approximately 99% of the HSA was depleted from standard samples as well as whole human serum (Figures 3 A and B).

In Figure 3 (A), 500µg samples of two unique lots of HSA standard protein were depleted with the SP-200 kit. Comparison of the intensity of the unbound albumin signal remaining in the sample with the depletion efficiency controls shows that the HSA was >99% depleted.

In Figure 3 (B), 10µL samples from two unique lots of human serum were depleted with the SP-200 kit. In the Western Blots of the SDS-PAGE analysis of the depleted serum samples, comparison of the intensity of the unbound albumin signal remaining in the sample with the depletion efficiency controls show that the HSA was >99% depleted. Furthermore, even the high molecular weight complex albumin species were also depleted.

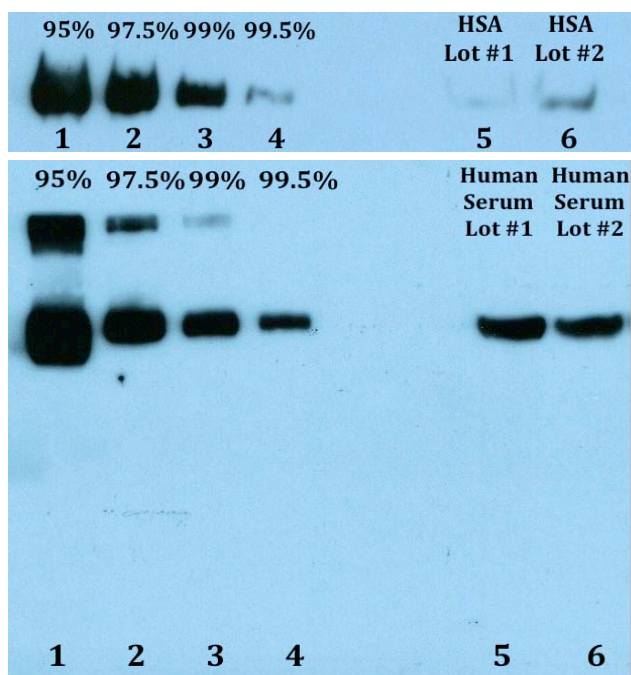


Figure 3. Western blots of 1D SDS-PAGE gels demonstrating the efficiency of the SP-200 ProteaPrep kit for the depletion of human serum albumin (HSA) from (A) standard HSA samples, and (B) 10µL human serum samples. >99% depletion of the HSA is demonstrated for each sample analyzed. Key for both gels: Lanes 1 to 4 = HSA depletion standards representing 95.0, 97.0, 99.0, and 99.5% depletion efficiency, respectively. Lanes 5 and 6 = depleted standard HSA (A) and human serum samples (B).

The compatibility of the ProteaPrep Albumin Depletion Sample Prep Kit for the removal of albumin from sera of other organisms was also studied. This technology was shown to effectively deplete serum albumin in samples from other organisms, and the results are summarized in Table 1.

Organism	Compatibility
Human	Yes
Rat	Yes
Mouse	Yes
Bovine	Yes
Pig	Yes
Goat	Yes
Horse	Yes
Rabbit	Yes
Monkey	Yes
Dog	Yes

Table 1. Compatibility of the SP-200 ProteaPrep Albumin Depletion Sample Prep kit for the depletion of serum albumin from other organisms

Conclusions

Albumin was depleted from human serum samples as well as HSA standards in less than 20 minutes using the ProteaPrep Albumin Depletion Kit. Results of initial studies indicate depletion efficiencies >99% for the selective removal of HSA from human serum samples. Additionally, this methodology has also been demonstrated to be effective for the selective depletion of albumin from the serum of other mammalian organisms

The ProteaPrep Albumin Depletion Sample Prep Kit is a powerful tool for researchers interested in the study of lower abundance proteins in serum.

Authors: Christopher A. Bolcato and Matthew J. Powell

Acknowledgements

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References

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