

Depletion of albumin in rodent sera using PureProteome™ albumin magnetic beads and sample concentration using Amicon® Ultra 2 mL centrifugal filters

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INTRODUCTION

Biomarkers offer important information about homeostasis, disease, response to drug treatments, and environmental stimuli. Sera are rich sources of biomarkers (biological indicator proteins, peptides, small molecules, etc.) and are easier to sample than other tissues. However, the complexity of serum and the presence of highly abundant proteins like albumin and immunoglobulin can mask less abundant species, hindering biomarker detection. PureProteome albumin magnetic beads remove more than 98% of albumin from human serum. Here, we demonstrate that PureProteome albumin magnetic beads may also be used to remove albumin from mouse, guinea pig and rat sera.

Depleted samples are often dilute, and may need concentration for downstream analyses. Therefore, we present a protocol for the convenient concentration of these samples using Amicon Ultra 2 mL centrifugal filters.

RESULTS

Optimization of the depletion protocol

Several factors, including amount of serum used, serum dilution factor, and total reaction volume, were analyzed during protocol optimization for mouse and rat serum depletion. Depleted serum samples were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), total protein assay, and enzyme-linked immunosorbent assay (ELISA) specific for mouse or rat albumin. As shown in Figure 1, the best depletion in mouse serum was obtained when using 12–25 μ L of total serum, diluted 1:4 or 1:8. Using higher serum concentration (50 μ L of total

serum) or lower concentration (6.5 μ L of total serum) reduced the efficiency of albumin removal. For rat serum, PureProteome beads were able to remove about 75% of the albumin, independent of amount of serum, the dilution or total reaction volume.

Quantification of albumin depletion

The albumin content in the sera of mice, rats, and guinea pigs, as in humans, represents about 50 to 70% of the total protein. ELISA results for mouse and rat sera indicate that PureProteome albumin and albumin/IgG beads removed more than 85% of the albumin in mouse and more than 75% of the albumin in rat serum (Figure 1). While there were no ELISA results available for the guinea pig samples, visual inspection of the stained gel (Figure 4) indicated similar depletion efficiency as in the mouse serum.

Concentration of depleted serum

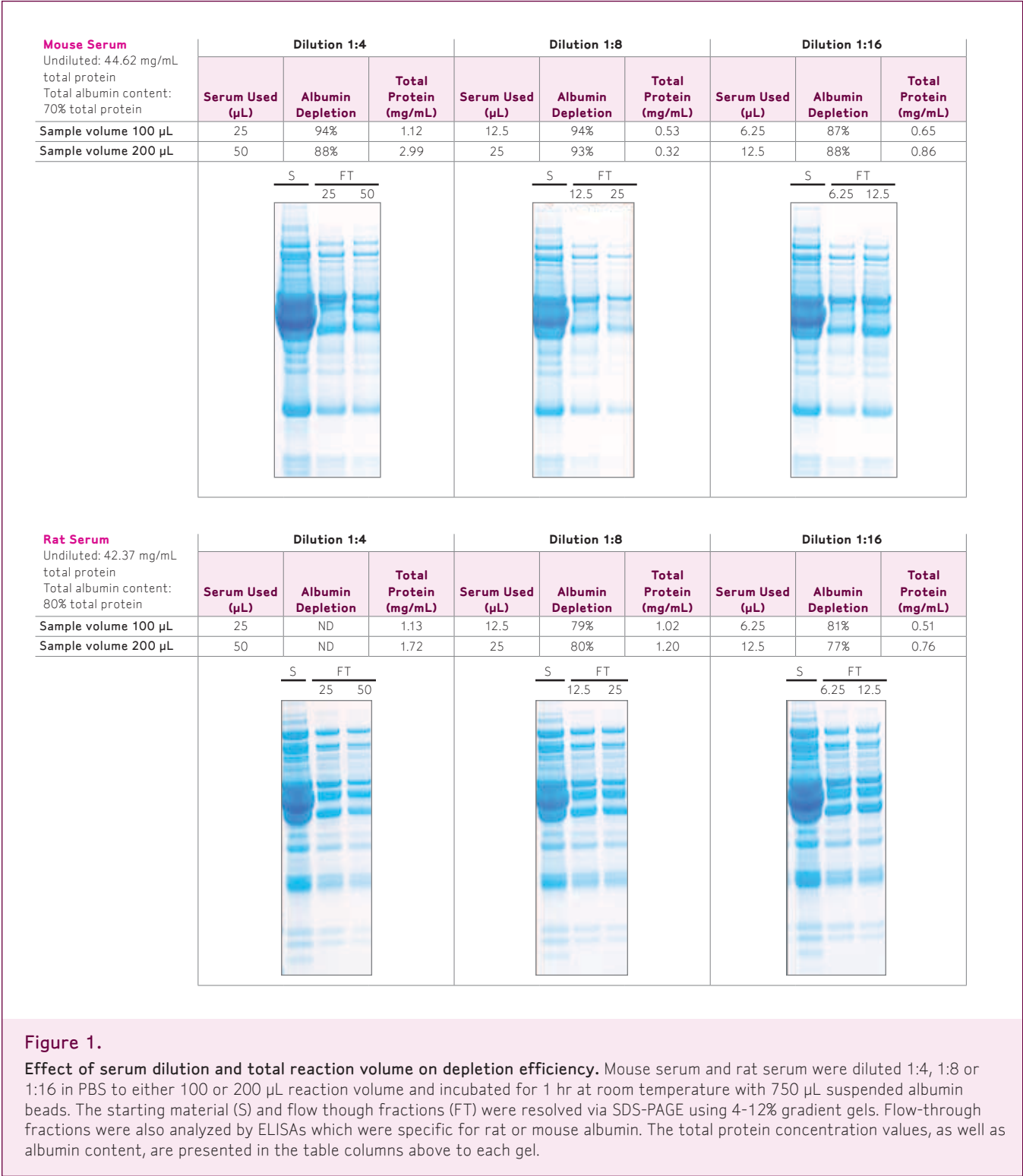
After removal of the most abundant protein(s), the total protein concentration of a serum sample is relatively low. Concentration of depleted serum samples was achieved using an Amicon Ultra 2 mL centrifugal filter with a 3,000 MWCO. Flow-through (FT) and wash (W) fractions can be concentrated before pooling, enabling fast concentration. Figure 3 shows that FT was concentrated from 158 μ L to 21 μ L in just 5 minutes. Washes, which were concentrated from 1.4 mL to 140 μ L, were centrifuged 20 min.

When the flow-through and washes were pooled (FT + W) prior to the concentration step, the devices were centrifuged for 30 min to obtain about 100 μ L of concentrated material (results shown in Figure 4 and Table 1).

CONCLUSIONS

Optimum depletion of rodent serum using PureProteome albumin magnetic beads was achieved using 12.5 μ L to 25 μ L of serum per 750 μ L of suspended beads. According to ELISA results, PureProteome albumin magnetic beads can deplete >75% of rat albumin and >85 % of mouse albumin in serum samples. Visual analysis of guinea pig samples

indicates a similar level of depletion as in the mouse serum. Further, the Amicon Ultra 2 mL filter with 3,000 MWCO is useful for concentrating the depleted serum samples by 3 to 10 times. It is important to mention that when wash fractions contain considerable amount of albumin, as in the case of rat serum, pooling of the wash fractions is not recommended.



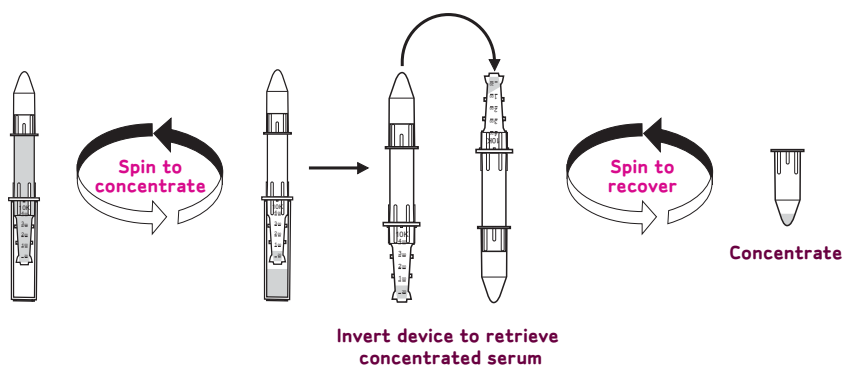
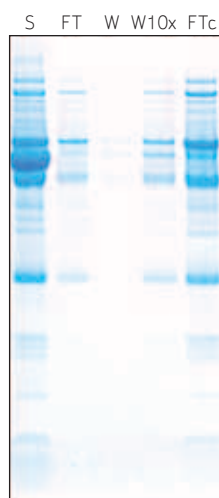


Figure 2.
Amicon Ultra 2 mL filter
protocol for concentration
and recovery.



FT = Flow-through before
concentration
FTc = Flow-through after
7X concentration
W = Wash fraction before
concentration
W10x = Wash fraction after
10X concentration

Figure 3.
Comparison of the flow-through and wash fractions
before and after concentration in Amicon Ultra 2 mL
filters. Depleted mouse serum (FT), as well as the 3 wash (W)
fractions, were independently concentrated 7 and 10 times,
respectively. 1 μ L of each sample was loaded on the gel.

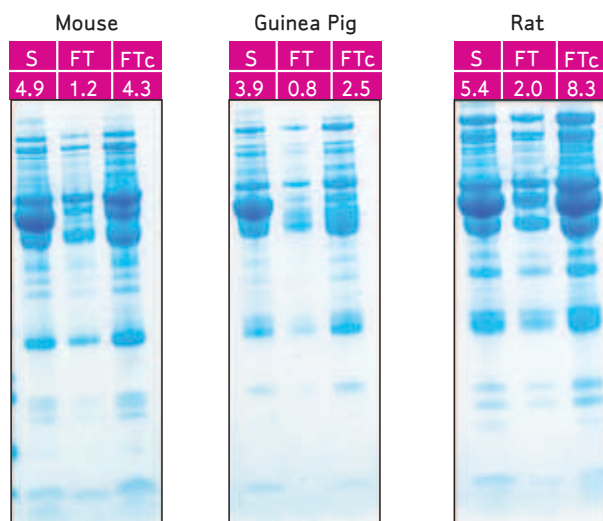


Figure 4.
Albumin depletion and serum concentration results.
Depleted mouse, guinea pig, and rat sera were concentrated
in Amicon Ultra 2 mL devices after pooling the flow-through
and washes (FT + 3 washes) for a total volume of about
1.7 mL. The total protein concentration of samples before
and after concentration is displayed on the top of the gel in
mg/mL. 1 μ L of each sample was loaded on gels.

S = Diluted starting material
FT = Depleted fraction before concentration
FTc= Depleted fraction pooled with wash after concentration

Table 1. Serum volume and concentration before and after centrifugation using Amicon Ultra 2 mL filters.

Serum type	Mouse	Guinea pig	Rat
Concentration of original serum sample (mg/mL)	45	31	42
Concentration of starting material (mg/mL) (S)	4.97	3.89	5.46
Volume of depleted serum + washes (FT + W)	1.6 mL	1.6 mL	1.59 mL
Protein concentration (mg/mL) of depleted serum (FT) before concentration	1.23	0.80	2.02
Final volume of depleted sample after concentration (FTc)	0.0924 mL	0.0979 mL	0.0785 mL
Protein concentration (mg/mL) of depleted serum after concentration (FTc)	4.29	2.55	8.38
Concentration factor of depleted sample	3.5	3.1	3.2

MATERIALS AND METHODS

Mouse (Millipore Cat. No. S25-10mL), rat (Millipore Cat. No. S24-100) or guinea pig (Sigma G9774) sera were diluted in PBS. Diluted samples (100-200 μ L final volume) were incubated for 1 hour with 750 μ L of equilibrated PureProteome albumin magnetic bead suspension (Millipore Cat. No. LSKMAGL10) using end-over-end mixing on a rotator. Depleted fractions (flow-through) were collected after capturing the beads with a magnetic stand (Millipore Cat. No. LSKMAGS08). Beads were washed 3 times with 500 μ L of PBS.

Sample concentration:

In several cases, the wash fractions were pooled with the depleted sample and samples were concentrated in Amicon Ultra 2 mL centrifugal devices with 3K MWCO (Millipore Cat. No. UFC200324PL). Devices were centrifuged for 5-30 min at 7,500 x g in a Jouan CR 422 centrifuge using a fixed angle rotor. Concentrated material was recovered by a reverse spin in the Amicon Ultra device for 1 min at 1000 x g (Figure 2).

Electrophoresis:

Samples were diluted in 1X gel loading buffer containing reducing agent and incubated at 70°C for 10 min. Reduced and denatured samples were subjected to SDS-PAGE, using 1 mm thick NuPAGE 4-12% gradient gels at 200 volts for 45 min. Gels were stained with colloidal Coomassie blue.

ELISA:

The efficiency of albumin depletion was determined using the ELISA kits from Innovative Research, specific for mouse (IRE-90ABL) or rat (IRE-60SBL) albumin. Plates were read at 450 nm on BioTek®-Synergy™ 2 microplate reader.

Protein Concentration Measurement:

Total protein concentration in starting material and depleted serum samples, before and after concentration, were determined by the Pierce® 660 nm protein assay kit (Thermo Fisher).

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