



## Application Note

# Immunoglobulin Depletion from Serum Samples Using PureProteome™ Protein A and Protein G Magnetic Beads

## INTRODUCTION

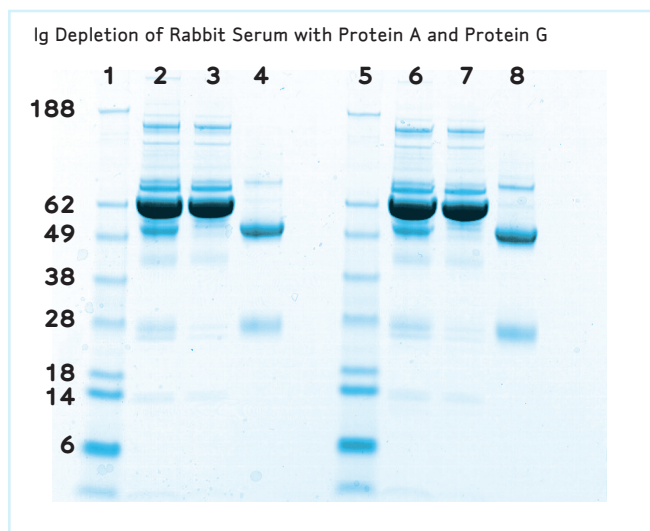
The development of new clinical sample preparation tools has been driven by accelerated clinical proteomics research focused on the identification of biomarkers that ultimately can be used by clinicians for disease diagnosis, prognosis and personalized therapy. The ability to diagnose a cancer patient by analyzing the proteins circulating in the blood before formation of a detectable tumor can increase the patient's chance of survival. Either an entire protein or one of its cleavage products, secreted from diseased cells into the bloodstream, can serve as a specific biomarker. Besides cancer diagnosis, the clinical application of biomarkers covers numerous other disease states including metabolic syndrome, diabetes, neurodegenerative disorders, with the types of clinical samples subjected to analysis varying widely.

Serum is frequently used as the sample source for biomarker discovery in multiple research focus areas. While serum may contain a wealth of proteomic information, analysis is hindered by the wide concentration range of proteins in serum. Abundantly expressed proteins such as immunoglobulins (Igs) and albumin are found at high concentrations (mg/mL), while proteins secreted from diseased cells are typically found at much lower concentrations (ng/mL - pg/mL). These high abundance proteins therefore can mask the lower abundance and potentially more informative proteins in analytic methods, such as two-dimensional gel electrophoresis and mass spectrometry. It is critical for biomarker research that the high abundance proteins are efficiently, reproducibly and specifically removed from serum samples to enable accurate analysis of lower abundance proteins.

There are currently several methods for depleting these higher abundance proteins from serum; prepared affinity or ion exchange columns. These options are expensive, require extensive sample dilution and can have high levels of non-specific binding. Recently, magnetic beads have grown in popularity as a matrix for protein fractionation. Since they can be immobilized instantly with a magnet and resuspended in any desired volume, the beads quickly yield fractionated samples that are ready for downstream analysis. Here we demonstrate the flexibility and scalability of PureProteome Protein A and Protein G magnetic beads in depleting Igs from serum samples. For efficient use of Protein A and G magnetic beads, several factors need to be considered, such as sample dilution, the amount of beads and the applicability of either Protein A or Protein G beads to a particular sample.

## OPTIMIZED PROTOCOL

A protocol was developed to achieve optimal percentage of Ig depletion from rabbit serum with minimal non-specific binding with either Protein A or Protein G beads. In this protocol, 10  $\mu\text{L}$  of rabbit serum was diluted with 190  $\mu\text{L}$  of phosphate-buffered saline (PBS) and incubated with 100  $\mu\text{L}$  of either Protein A or Protein G bead suspension. The percentage of Ig depletion determined by enzyme-linked immunosorbent assay (ELISA) analysis was greater than 98% for both types of beads. As shown in Figure 1, there is no detectable Ig remaining in the depleted fractions and minimal non-specific binding in the bound fractions. The following sections summarize experiments performed to optimize Ig depletion from different sera.



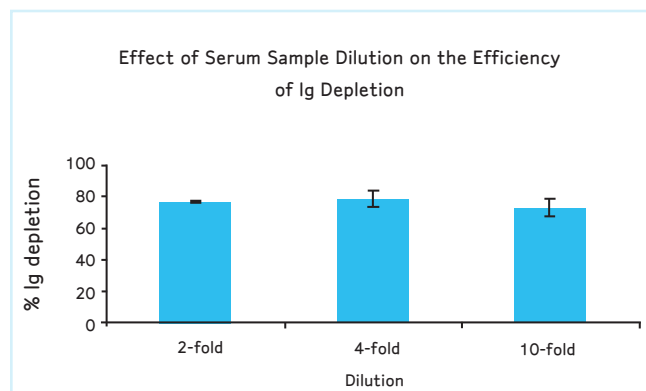
**Figure 1.**

Ig depletion of rabbit serum with 100  $\mu\text{L}$  of Protein A (lanes 2-4) and Protein G (lanes 6-8) bead suspension. Samples of the input material, depleted flow-through and bound fractions were visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Lanes 1 and 5; molecular weight markers, 2 and 6; input material, 3 and 7; flow-through (Ig-depleted) serum, and 4 and 8; bound Ig fraction.

## OPTIMIZATION GUIDE

### Sample Dilution

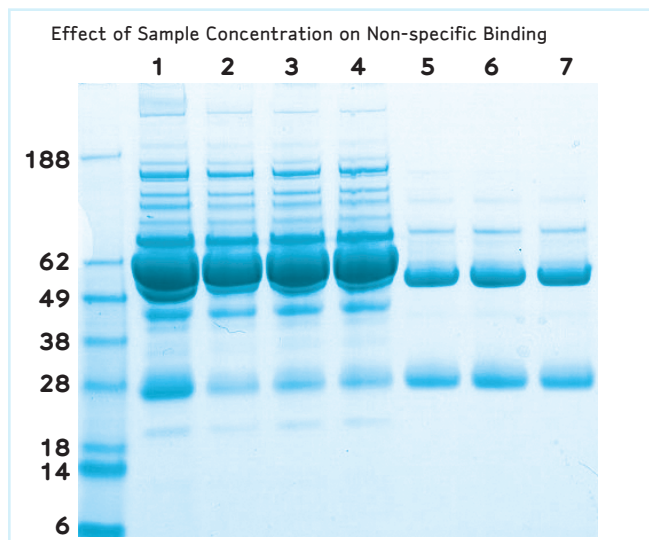
For several downstream applications requiring depleted serum, the processing volume may be critical. Starting serum samples that are diluted prior to application on a depletion column can affect the accuracy of the downstream analytical method. The effect of serum dilution on the efficiency of depletion was investigated by diluting 25  $\mu\text{L}$  of human serum 1:2, 1:4 or 1:10 with PBS prior to incubation with 100  $\mu\text{L}$  of Protein G bead suspension. The percentage Ig depletion was determined for each diluted sample. As shown in Figure 2, efficient Ig depletion was relatively independent of sample dilution factor — there was only a slight decrease in the percentage Ig depletion when the samples were diluted 10-fold. These data demonstrate that the use of magnetic beads for Ig depletion offers researchers the ability to minimally dilute the starting serum sample while still achieving a high percentage of Ig depletion.



**Figure 2.**

Bar graph showing the percent of Ig bound to beads as a fraction of total Ig in original sample, as a function of fold dilution of sample being depleted.

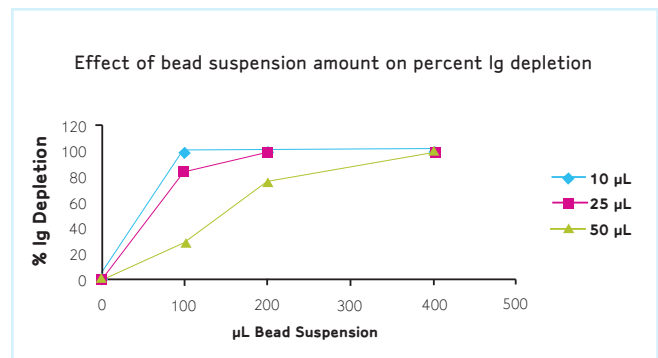
In some instances it may be of interest to study the bound Ig fraction. The effect of sample concentration or dilution on non-specific protein binding to the beads was also evaluated. Both the depleted flow-through and bound fractions were compared by SDS-PAGE analysis. As shown in lanes 5-7 of Figure 3, the bound fractions of the three dilutions have similar profiles demonstrating that the more concentrated samples do not have higher levels of serum non-specific protein binding than the more dilute samples. Therefore, dilution is not required in order to achieve efficient depletion or to minimize non-specific protein binding.



**Figure 3.** SDS-PAGE analysis of input sample, depleted, and bead-eluted fractions of Ig-depleted serum with respect to sample dilution factor. Lane 1; input material, lanes 2-4; depleted samples, 5-7; eluted fractions. Lanes 2 and 5; 2-fold dilution; lanes 3 and 6; 4-fold dilution; lanes 4 and 7; 10-fold dilution.

## Bead and Serum Volume

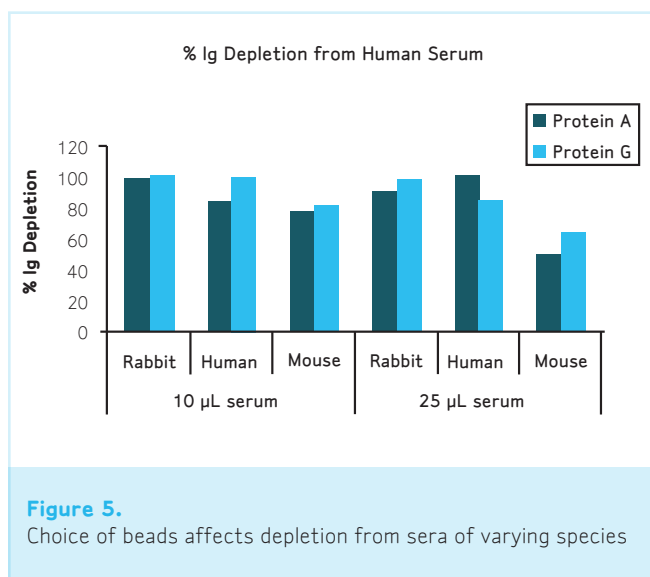
The proportion of beads to serum can also affect the efficiency of a serum depletion experiment. To determine the amount of beads necessary for optimal serum depletion, 100  $\mu\text{L}$  of the bead suspension was incubated with 10  $\mu\text{L}$ , 25  $\mu\text{L}$  and 50  $\mu\text{L}$  of human serum diluted to 200  $\mu\text{L}$  with PBS. The percentage Ig depletion was calculated by ELISA analysis. As shown in Figure 4, higher amounts of beads are required to deplete increasing amounts of serum. The optimal percentage of Ig depletion can be achieved with 100  $\mu\text{L}$  of the bead suspension for 10  $\mu\text{L}$  of serum, 200  $\mu\text{L}$  of the bead suspension for 25  $\mu\text{L}$  serum and 400  $\mu\text{L}$  of the bead suspension for 50  $\mu\text{L}$  serum. These values are based on human serum; so there may be some differences in the percentage of Ig depletion based on the concentration of the Igs in other serum samples.



**Figure 4.** An ELISA assay was used to determine the percentage of IgG depletion of three different amounts of serum by increasing amounts of bead suspension. (diamonds) 10  $\mu\text{L}$  serum, (squares) 25  $\mu\text{L}$  serum, (triangles) 50  $\mu\text{L}$  serum.

## Selection of Protein A or Protein G

The binding affinities of Igs for Protein A and Protein G varies depending on the species of the serum sample. Both Protein A and Protein G can deplete Ig from three of the most common serum species used in biomarker research: mouse, rabbit, and human. To assess the best bead for each serum type, either 10  $\mu$ L or 25  $\mu$ L of serum was diluted to 200  $\mu$ L with PBS and incubated with 100  $\mu$ L of Protein A and Protein G bead suspensions. The depleted fractions were then analyzed by ELISA assay. As shown in Figure 5, both Protein A and Protein G deplete Ig with the same efficiency for rabbit serum. However, Protein G is more effective than Protein A at depleting Ig from human and mouse sera.



**Figure 5.** Choice of beads affects depletion from sera of varying species

## CONCLUSIONS

PureProteome Protein A and Protein G magnetic beads have proven effective in depleting Ig from serum samples and provide an alternative method to current depletion techniques. After optimizing depletion protocols for bead type, sample dilution, and bead suspension amount, magnetic beads offer researchers a rapid and reproducible method for selective and scalable depletion of Igs with minimal sample dilution.

## MATERIALS AND METHODS

### Ig Depletion:

100  $\mu$ L of the bead suspension was washed three times with 500  $\mu$ L of PBS. Serum samples, diluted in PBS, were then added to the beads and incubated at room temperature for 30 minutes with constant mixing. The depleted samples were collected and the beads were washed three times with 500  $\mu$ L of PBS. Bound Ig was then eluted from the beads by the addition of 50  $\mu$ L of 0.2 M glycine (pH 2.5).

### Electrophoresis:

Samples were diluted in 1X Sample Buffer with reducing agent and incubated at 70  $^{\circ}$ C for 10 minutes. The prepared samples were subjected to SDS-PAGE (4 - 12% gradient gels) using commercially prepared, 10-well, 1 mm thick minigels (Invitrogen NuPAGE<sup>®</sup> gels), run at 200 V for 35 minutes. The gels were removed from the cassettes, washed in Milli-Q<sup>®</sup> water three times and stained with colloidal Coomassie Blue.

### Determining IgG Depletion

To calculate the percentage Ig depletion, the flow-through fractions were analyzed using a Human IgG (Zeptometrix catalog # 0801182), Rabbit IgG (Zeptometrix catalog # 0801202), or Mouse IgG (Zeptometrix catalog # 0801180) ELISA assay.

### Buffers:

PBS (0.01 M phosphate buffered saline; 138 mM NaCl, 27 mM KCl, pH 7.4)



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