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Chemistry beyond the ordinary

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ProMax Albumin Removal Kit

Protein Enrichment through Albumin Removal based on patented BioMag® particle technology.

Catalog Number 24351

Introduction:
Changes that occur in serum and plasma proteins have long been recognized as a way to investigate and monitor physiological changes. This rich source of information does however present challenges for most of the analytical methods used. One of the reasons for this is that one-dimensional and two-dimensional electrophoresis, high performance liquid chromatography, and mass spectroscopy have a limited dynamic range for the amount of protein mass that can be loaded and resolved. In addition, greater than 50% of the protein in serum is represented by albumin. The presence of this and other highly abundant proteins lowers the detection threshold for the proteins of interest. Therefore a methodology is needed that can effectively partition the highly abundant and less abundant proteins.

There are currently several methods that allow serum albumin removal. Cibacron-blue coupled to chromatography supports has been widely used but it lacks specificity. Anti-albumin antibodies are also being used in immuno-affinity systems. These systems generally have good specificity, but are expensive and have the potential of introducing proteins from the affinity separation media into the sample. Also, because many of these systems are based on column chromatography, enrichment of the less abundant proteins is often accomplished with an increase in sample volume, necessitating an additional protein concentration step.

The ProMax Albumin Removal Kit is based on patented BioMag superparamagnetic particle technology providing a rapid and simple protocol for serum albumin removal. The magnetically responsive ProMax Albumin Removal Particles supplied in the kit, in combination with specific buffer conditions, allows the binding and release of the less abundant proteins in serum while minimizing the binding of albumin. The ProMax protocol is scalable and can be used in conjunction with the ProMax Serum IgG Removal Kit (Cat. #24352-1).

Figure 1:

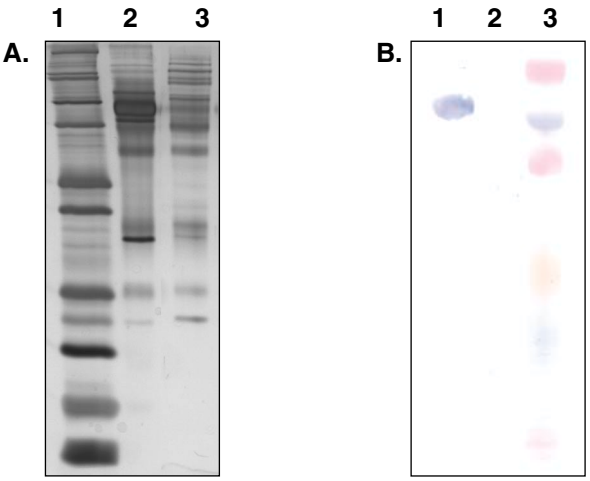

Figure 1. SDS-PAGE analysis and Western Blot showing enrichment of lower abundance proteins and depletion of albumin.

Panel A shows a silver stained SDS-PAGE gel. Lane 1, Molecular Weight Markers; Lane 2, untreated normal human serum; Lane 3, serum treated with ProMax Albumin Removal Particles. Both Lanes 2 and 3 were loaded with the same amount of protein.

Panel B shows the depletion of albumin by Western Blot. Lane 1, normal serum; Lane 2, serum treated with the ProMax Albumin Removal Kit; Lane 3, Molecular Weight Markers. Mouse anti-albumin was the primary antibody and the signal was visualized using an anti-mouse horseradish peroxidase conjugate and TMB as the chromagen. Both Lanes 1 and 2 were loaded with equal amounts of protein. Lane 2 shows that nearly all of the albumin has been depleted from the sample.

Figure 2:

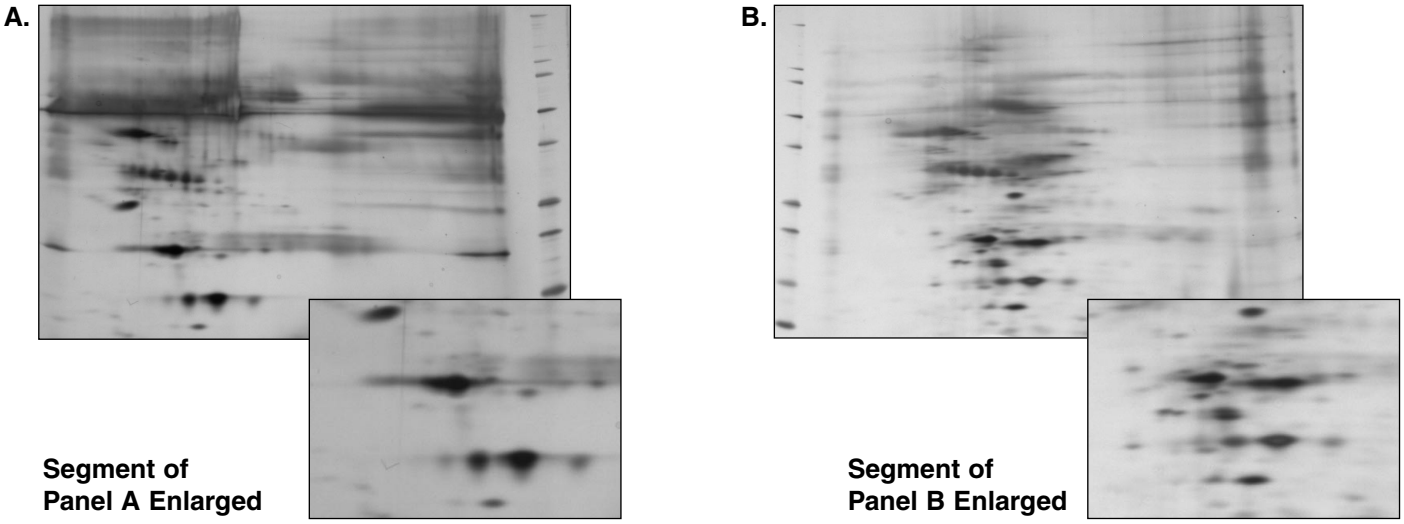

Figure 2. 2-D Gel analysis of serum treated with ProMax Albumin Removal System. Equal amounts of protein were analyzed by 2-D gels using 3-10% IEF gradients in the first dimension followed by 4-20% SDS-PAGE for the second dimension and then silver stained. Panel A shows untreated normal serum, Panel B serum after treatment with the ProMax Albumin Removal Kit. Comparison of the two panels shows a significant enrichment of less abundant proteins in the treated samples.

Kit Components:	**25 Reactions**
ProMax Albumin Removal Particles	1.5ml
ProMax Albumin Removal Binding/Wash Buffer	45.0ml
ProMax Albumin Removal Elution Buffer	3.0ml

Procedure:

1. Add 35µl of ProMax Albumin Removal Binding/Wash buffer to a microcentrifuge tube or well of a microtiter plate for each sample to be processed.
2. Add 10µl of serum to the Binding/Wash buffer and mix thoroughly[1].
3. Resuspend the ProMax Particles thoroughly by shaking or vortexing. To each well or microcentrifuge tube containing diluted serum, add 50µl of ProMax Particles. Mix thoroughly and then incubate for 10 minutes at room temperature with constant mixing.
4. Pellet the ProMax Particles by magnetic separation. Remove the supernatant, which will contain albumin.
5. Wash the ProMax Particles three times by resuspending the pellets in 500µl of Binding/Wash Buffer,thoroughly mixing until the ProMax Particles are completely resuspended, then pelleting the ProMax Particles via magnetic separation.
6. After the third wash resuspend the ProMax Particles in 50µl to 100µl of ProMax Elution Buffer.[2]
7. Incubate at room temperature for 10 minutes with constant mixing.
8. Magnetically separate the ProMax Particles and transfer the supernatant liquid to a fresh microcentrifuge tube or microtiter plate well.[3] The supernatant will contain the proteins of interest.
9. If ProMax Particles are inadvertently carried over into the removed supernatant, repeat the magnetic separation on the collected supernatant to remove the ProMax Particles.

Notes:

[1]Ten microliters of human serum will contain approximately 700µg of total protein. Of that, approximately 400µg will be human serum albumin. The amount of protein in serum can vary. Each user should optimize the amount serum put in each reaction. Overloading of the system may result in carry over of albumin into the low abundant protein fraction.

[2]The volume of elution buffer should also be optimized by the user. The protein concentration of the eluted proteins will differ in accordance with the starting protein concentration of the sample. If desired, the user may elute the proteins in volumes as small as 10µl. While it is often desirable to elute in as small a volume as possible, smaller elution volumes may result in lower yields. Using the protocol above, the user can expect a typical elution to contain 50µg to 100µg of serum protein.

[3]The samples are eluted in a salt containing buffer and may need to be de-salted prior to analysis by electrophoresis or mass spec. Desalting may not be needed if the sample is sufficiently diluted prior to analysis. If desired, desalting can be accomplished by precipitating the proteins. Dilute the samples in at least 10 volumes of acetone, incubate for 10 minutes on dry ice, then centrifuge at 10,000 x g for 10 minutes, and briefly dry the pellet before resuspending. Acetone precipitation is also a convenient way to concentrate the protein sample. Desalting spin columns can also be used. Refer to the manufacturers instructions on the use of desalting columns. Note that desalting and/or concentration has the potential to alter the protein profile of the sample and the user is encouraged to consider this when choosing a desalting or concentration method.

Ordering Information:

Catalog #	Description	Size
24351-1	ProMax Albumin Removal Kit	1kit
8MB4112S-1	BioMag Solo-Sep Microcentrifuge Tube Separator	1 magnet
8MB4111S-1	BioMag Multi-6 Microcentrifuge Tube Separator	1 magnet
84106S-1	BioMag Multi-32 Microcentrifuge Tube Separator	1 magnet
8MB4109S-1	BioMag 96-Well Plate Separator	1 magnet
85072S-1	BioMag 96-Well Plate Side Pull Magnetic Separator	1 magnet

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