Product Data Sheet 658

BioMag® ProMax Albumin Removal Kit

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B E A D S ● A B O V E T H E R E S T ™

DESCRIPTION

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 BioMag® ProMax Albumin Removal Kit is based on patented BioMag® superparamagnetic particle technology, and this provides a rapid and simple protocol for serum albumin removal. The magnetically responsive BioMag® ProMax Albumin Removal Particles supplied in the kit, in combination with specific buffer conditions, allow the binding and release of less abundant proteins in serum, while minimizing the binding of albumin, so that it may be washed away.

CHARACTERISTICS

Mean Diameter:	~1.5µm
Particle Concentration:	5 mg/mL
Number of Reactions per Kit:	25

MATERIAL

Material Supplied

- BioMag® ProMax Albumin Removal particles: 1.5mL
- ProMax Albumin Removal Binding / Wash Buffer: 45mL
- ProMax Albumin Removal Elution Buffer: 3mL

Material Required

- Microcentrifuge tubes
- Magnetic separator

Figure 1:

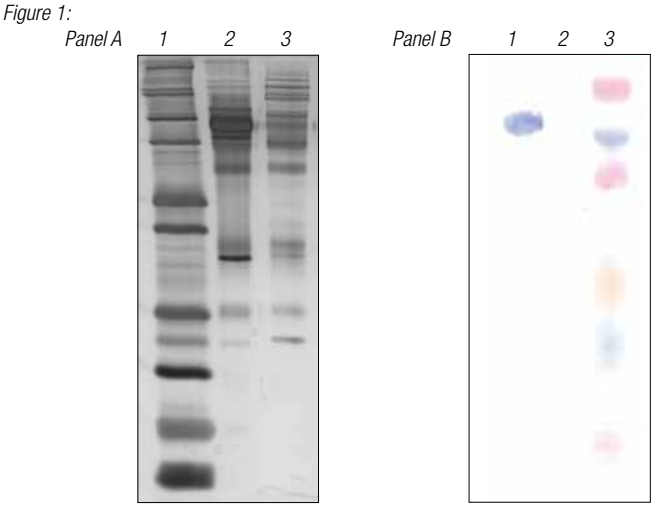

Figure 1. SDS-PAGE analysis and Western Blot showing enrichment of lower abundance proteins and depletion of albumin.

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 BioMag® 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 BioMag® 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:

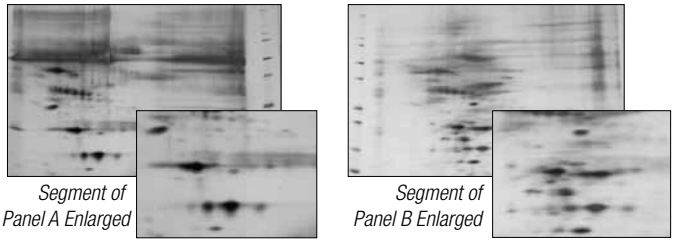

Figure 2. 2-D Gel analysis of serum treated with BioMag® ProMax Albumin Removal System. *Equal amounts of protein were analyzed by 2-D gels using 3-10% IEF gradients in the first dimension followed by 4-20% SDS-PAGE for the second dimension and then silver stained. Panel A shows untreated normal serum;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

PROCEDURE

Researchers are advised to optimize the use of particles in any application, as procedures designed by other manufacturers may not be ideal.

1. Add 35µL of 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.
3. Resuspend the particles thoroughly be shaking or vortexing. To each well or microcentrifuge tube containing diluted serum, add 50µL of BioMag® ProMax particles. Mix thoroughly and then incubate for 10 minutes at room temperature with constant mixing.
4. Pellet the particles by magnetic separation. Remove the supernatant, which will contain albumin.
5. Wash the particles 3 times by resuspending the pellets in 500µL of Binding / Wash Buffer, thoroughly mixing until the particles are completely resuspended, then pelleting the particles via magnetic separation.
6. After the third wash, resuspend the particles in 50-100µL of Elution Buffer.
7. Incubate at room temperature for 10 minutes with constant mixing.
8. Magnetically separate the particles and transfer the supernatant liquid to a fresh microcentrifuge tube or microtiter plate well. The supernatant will contain the proteins of interest.
9. If particles are inadvertently carried over into the removed supernatant, repeat the magnetic separation on the collected supernatant to capture the 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 of 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 50-100µg 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 spectrometry. 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 acetone, at least 10 times the volume of your sample, and incubate for 10 minutes on dry ice, then centrifuge at 10,000 X G for 10 minutes, and briefly dry the pellet before suspending. Acetone precipitation is also a convenient way to concentrate the protein sample. Desalting spin columns can also be used. Refer to the manufacturer's 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.

STORAGE AND STABILITY

Store at 2-8˚C. Freezing, drying, or centrifuging particles may result in irreversible aggregation and loss of binding activity.

SAFETY

This particle suspension contains sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Material Safety Data Sheet for more information.

This product is for research use only and is not intended for use in humans or for *in vitro* diagnostic use.

ORDERING INFORMATION

Cat. Code	Description	Size
BP658	BioMag® ProMax Albumin Removal Kit	1 kit

Order online anytime at **www.bangslabs.com.**