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TECHNICAL DATA SHEET 659

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ProMax Serum IgG Removal Kit Protein Enrichment through serum IgG removal based on patented BioMag[®] superparamagnetic particle technology.

Catalog Number 24352

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. This limited dynamic range effects the resolution of less abundant proteins. Albumin and IgG together can represent over 75% of the total protein in serum, masking the ability to detect less abundant proteins of interest. Albumin represents 50-70% of the total protein in serum and IgG can represent 10-20% of the total protein. If the majority of these two proteins can be removed from serum samples, a large increase in the resolution of less abundant proteins can be obtained.

The ProMax Serum IgG Removal Kit is based on patented BioMag[®] superparamagnetic particle technology. The ProMax IgG Removal Particles, in combination with specific buffer conditions, bind the IgG from the serum enabling it to be depleted from the sample. Using magnetically responsive particles for depletion of IgG has advantages over other systems. Removal of IgG with the ProMax IgG Removal Kit is a rapid and simple procedure that requires no pretreatment of the sample. In addition, the ProMax system does not require the use of time consuming columns or centrifugation. The ProMax protocol is scalable and can be used in conjunction with the ProMax Albumin Removal Kit (Cat. #24351-1).

Figure 1:

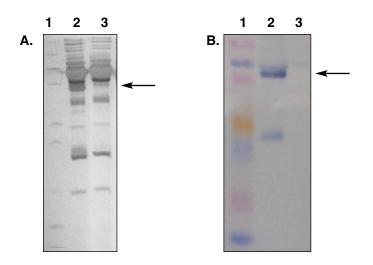


Figure 1. SDS-PAGE analysis and Western Blot showing depletion of IgG from human serum.

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 IgG Removal Kit. Lanes 2 and 3 were loaded with the same amount of protein. The arrow indicates the location of the major IgG band. Panel B shows a Western Blot with MW Markers in Lane 1, normal serum in Lane 2 and serum treated with the ProMax IgG Removal Kit in Lane 3. IgG was visualized using a goat anti-human horseradish peroxidase conjugate and TMB as the chromagen. Both Lanes 2 and 3 were loaded with equal amounts of protein. Lane 3 shows that nearly all the IgG has been depleted from the sample.

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Kit Components:	10 rxns
ProMax IgG Removal Particles	450µl

ProMax IgG Removal Binding/Wash Buffer 650µl

Procedure:

- 1. Add 59µl of ProMax IgG Removal Binding/Wash Buffer to a microcentrifuge tube or well of a microtiter plate for each sample to be processed.
- 2. Add 1µl of serum to the ProMax IgG Removal Binding/Wash Buffer and mix thoroughly¹.
- 3. Resuspend the ProMax IgG Removal Particles thoroughly by shaking or vortexing. To each well or microcentrifuge tube add 40µl of ProMax particles to the diluted serum. Mix thoroughly and then incubate for 10 minutes at room temperature with constant mixing.
- 4. Pellet the ProMax IgG Removal Particles by magnetic separation. Collect the supernatant to a fresh tube or well. This is the IgG depleted fraction and is ready for downstream processing or analysis.²

Notes:

¹Normal human serum contains 10 to 20% of IgG protein. The optimal amount of protein in serum can vary and should be optimized by each user. Overloading of the system may result in greater amounts of IgG in the depleted fraction. The system may be scaled up for larger volumes of serum.

²If the user desires to remove albumin from the IgG depleted fraction using the Polysciences ProMax Albumin Removal Kit (Cat. #24351), it will be necessary to adjust the pH to 6.0 prior to exposure of the IgG depleted sample to the ProMax Albumin removal particles. This can be accomplished by adding a small volume of concentrated HCI to the fraction. The user is encouraged to optimize this step.

Ordering Information:

Catalog #	Description	Size
24352-1	ProMax Serum IgG Removal Kit	1 kit
24351-1	ProMax Albumin Removal Kit	1 kit
8MB4112S-1 8MB4111S-1 84106S-1 8MB4109S-1 85072S-1	BioMag Solo-Sep Microcentrifuge Tube Separator BioMag Multi-6 Microcentrifuge Tube Separator BioMag Multi-32 Microcentrifuge Tube Separator BioMag 96-Well Plate Separator BioMag 96-Well Plate Side Pull Magnetic Separator	1 magnet 1 magnet 1 magnet 1 magnet 1 magnet

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