

SAMPLE PREPARATION

Aurum™ Serum Protein Mini Kit

Expression Proteomics // Tools for Protein Separation and Analysis

- Removes up to 90% of albumin and IgG
- Quick and easy single-column protocol
- Treated samples ready for direct 2-D analysis

Enhance Detection of Low-Abundance Serum Proteins on 2-D Gels

Bio-Rad Expression Proteomics

Bio-Rad's approach to expression proteomics focuses on three technologies: sample preparation, two-dimensional (2-D) electrophoresis, and imaging and analysis. Each technology is ideally suited to a 2-D gel electrophoresis approach to analysis of sample proteins.

High-quality sample preparation prior to 2-D electrophoresis is critical for producing meaningful, reproducible results. Bio-Rad offers the most comprehensive suite of products available for general-purpose sample cleanup to enhance resolution, and for sample fractionation to reduce sample complexity.

Simultaneous Removal of Albumin and IgG

The isolation of lower-abundance proteins from serum or plasma is often complicated by the presence of albumin and immunoglobulin G (IgG). Albumin is the most abundant protein (~60–70%), while IgG is the second most abundant protein (~10–20%) in serum. These two proteins effectively act as major contaminants, masking the presence of many comigrating proteins, as well as limiting the amount of total serum protein that can be resolved on a 2-D gel. As part of Bio-Rad's solutions for general-purpose cleanup, the Aurum serum protein mini kit was specifically designed to maximize resolution of proteins of interest, vastly improving and supporting protein discovery and identification.

In the past, removal of albumin and IgG usually required separate methods, a costly and time-consuming approach. The Aurum serum protein kit consists of Micro Bio-Spin™ columns filled with a mixture of Affi-Gel® Blue and Affi-Gel protein A resins. This resin blend allows selective binding and simultaneous removal of both albumin and IgG from serum or plasma samples prior to 2-D gel electrophoresis, thereby minimizing handling steps and increasing efficiency. By using only one column for purification, the process retains precious samples for subsequent gel analysis.

By removing up to 90% of albumin and IgG, the Aurum serum protein kit significantly improves the resolution of polypeptide spots on 2-D gels. Treated samples are ready for 2-D analysis with no additional purification.



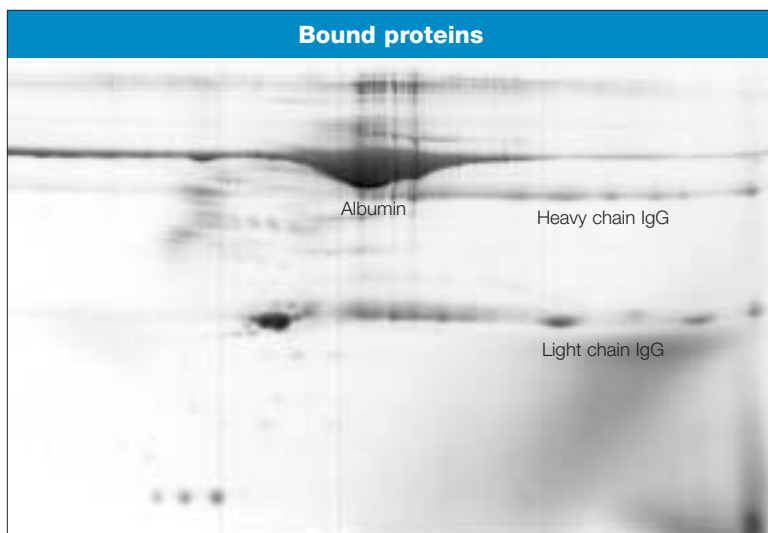
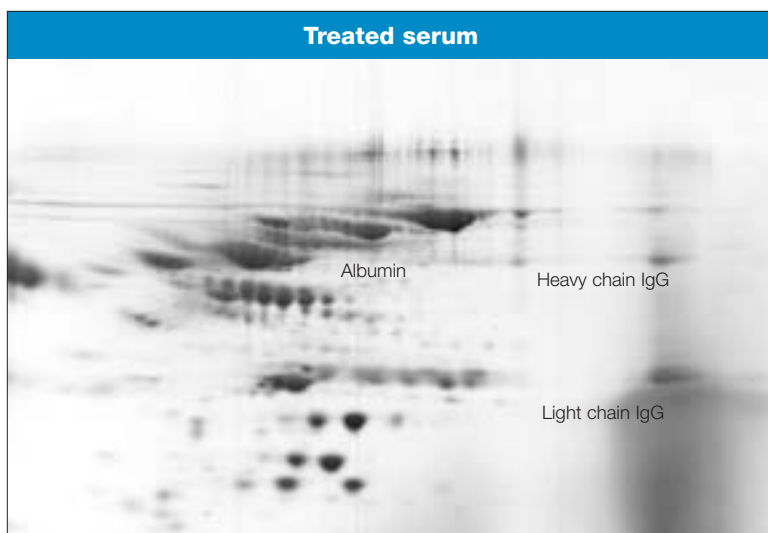
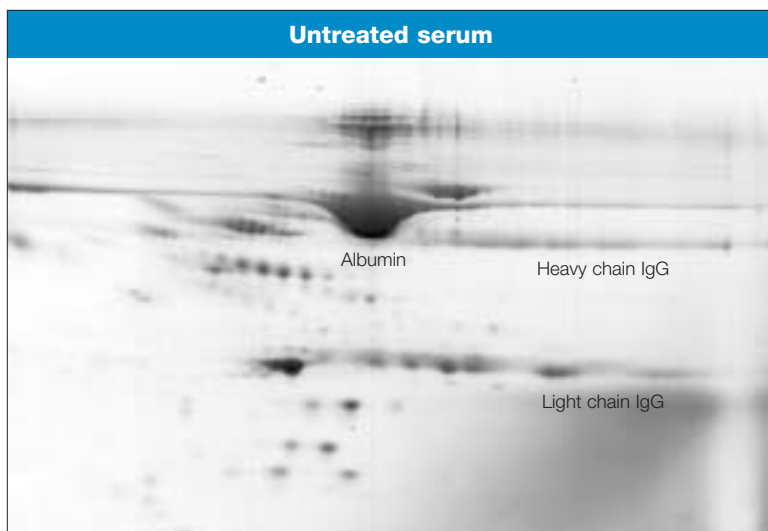
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Enhance Your 2-D Results

Sample purification with the Aurum serum protein mini kit ensures effective removal of albumin and IgG. The removal of these major proteins reduces the total serum protein by 70% or more, so that 3–4 times more of the enriched serum sample can be loaded. Low-abundance proteins can thus be visualized; in addition, proteins that comigrate with albumin and IgG are better resolved.

Because the Aurum serum protein mini kit utilizes Cibacron Blue technology, some nonspecific binding of other serum proteins may occur. However, this can add an extra dimension to your analysis. All proteins bound to the column can be resolved with a simple elution using ReadyPrep™ sequential extraction reagent 3. The proteins are stripped from the column and can be loaded directly onto an IPG strip for isoelectric focusing (IEF) analysis.

Total protein (1.32 mg) was loaded onto an Aurum serum protein mini column. Then 200 µg total protein from each fraction (untreated serum, treated serum, bound proteins) was loaded onto ReadyStrip™ IPG strips (pH 3–10, 11 cm). Criterion™ precast gels (8–16%, 4% stacking gel, Tris-HCl, 13.3 x 8.7 cm) were run at 200 V for 1 hr in a Criterion Dodeca™ cell and stained with Bio-Safe™ Coomassie stain. Images were acquired on the GS-800™ calibrated densitometer using PDQuest™ 2-D analysis software.

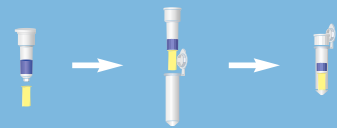
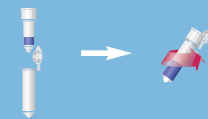


Protocol Overview

For complete protocol, consult instruction manual.

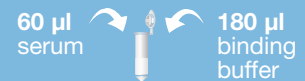
Column Setup

1. Place serum protein column in a test tube for 5 min to allow resin to settle.
2. Remove cap, break tip from column, and return column to test tube to start gravity flow in column.
3. Wash column with 1 ml of serum protein binding buffer using gravity flow. Repeat.
4. Place column in empty 2.0 ml collection tube and centrifuge for 20 sec at 10,000 x g to dry resin bed. **Discard collection tube.**
5. Place a yellow column tip onto bottom of column and place into a clean 2.0 ml collection tube labeled "unbound".



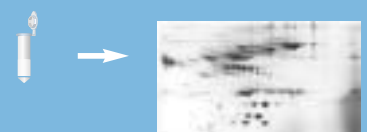
Sample Binding and Purification

6. In a separate tube, prepare sample by diluting 60 μ l serum or plasma with 180 μ l of serum protein binding buffer.
7. Add 200 μ l of diluted serum to top of resin bed in column.
8. Gently vortex column and repeat after 5 and 10 min. **Allow column to sit an additional 5 min.**



Collection of Purified Samples

9. Remove yellow tip from column and return column to tube. Centrifuge column for 20 sec at 10,000 x g, collecting protein fraction in "unbound" collection tube.
10. Using same collection tube, wash column with 200 μ l of serum protein binding buffer.
11. Centrifuge column for 20 sec at 10,000 x g, collecting protein fraction in same "unbound" collection tube. Discard serum protein column.
12. The combined fractions contain the albumin- and IgG-depleted serum or plasma sample. The sample is now ready for gel analysis.



Specifications

Method	Centrifuge-assisted affinity chromatography
Albumin removal	≤3 mg
IgG removal	≤1 mg
Preparation time	<30 min
Sample volume	≤250 µl
Stability	1 year at 4°C

Ordering Information

Catalog #	Description
732-6713	Aurum Serum Protein Mini Kit, 2 preps, includes 2 serum protein columns, 2 clear 12 x 75 mm polystyrene tubes, 6 collection tubes, 10 column tips, 15 ml binding buffer, protocol overview, instructions
732-6701	Aurum Serum Protein Mini Kit, 10 preps, includes 10 serum protein columns, 10 clear 12 x 75 mm polystyrene tubes, 30 sample collection tubes, 10 column tips, 50 ml binding buffer, protocol overview, instructions
161-0363	Precision Plus Protein™ Unstained Standards, 100 applications
161-0374	Precision Plus Protein Dual Color Standards, 50 applications
163-2014	ReadyStrip IPG Strips, 11 cm, pH 3–10, 12
163-2104	ReadyPrep Sequential Extraction Kit Reagent 3, 10 ml, 5 M urea, 2 M thiourea, 2% CHAPS, 2% SB 3-10, 40 mM Tris, 0.2% Bio-Lyte® 3/10 ampholyte
163-2106	ReadyPrep 2-D Starter Kit Rehydration/Sample Buffer, 1 vial, lyophilized, reconstitutes to 10 ml
165-4000	PROTEAN® IEF System, complete, includes basic unit, 17, 11, and 7 cm focusing trays with lids, 1 pack each of 17, 11, and 7 cm rehydration/equilibration trays with lids, 2 pairs of forceps, pack of electrode wicks, mineral oil, cleaning brushes
165-6001	Criterion Cell, includes tank, lid with power cables, 3 sample loading guides (12+2 well, 18-well, 26-well), instructions
345-0105	Criterion Tris-HCl Gel, 8–16%, 11 cm, IPG+1 well comb
500-0122	RC DC™ Protein Assay Kit II, includes RC™ reagents package, DC™ protein assay reagents package, bovine serum albumin standard; 500 standard assays

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