
Aurum™ Serum Protein Mini Kit

Instruction Manual

For technical service, call your local Bio-Rad office, or
in the US, call 1-800-4BIORAD (1-800-424-6723)

BIO-RAD

Table of Contents

Section 1	Introduction	1
Section 2	Kit Components	2
Section 3	Storage Conditions	2
Section 4	Necessary Supplies	2
Section 5	Guidelines for Using the Aurum Serum Protein Mini Kit	4
Section 6	Protocol	5
Section 7	Troubleshooting Guide	8
Section 8	References	9
Section 9	Ordering Information	10

Section 1

Introduction

The Aurum serum protein mini kit contains Micro Bio-Spin™ columns filled with a mixture of Affi-Gel® Blue and Affi-Gel protein A. This resin blend allows for the simultaneous removal of both albumin and immunoglobulin (IgG) in a single step from serum or plasma samples prior to two-dimensional (2-D) polyacrylamide gel electrophoresis (PAGE).

Proteins in serum and other biological fluids are difficult to resolve by 2-D PAGE, largely due to the abundance of serum albumin and IgG. In human serum, albumin constitutes 50–70% of the total protein and IgG constitutes 10–25%. The presence of these proteins obscures other proteins in the gel and limits the amount of proteins in the serum that can be resolved by 2-D analysis. Furthermore, these proteins have wide pI and molecular weight ranges, further reducing resolution and masking many proteins of potential interest. Theoretically, removing 90% of the albumin and IgG from serum should reduce the protein load by 70–80%, thus allowing the application of 3–4 times more serum and at the same time significantly improving the resolution of polypeptide spots on 2-D gels.

Affi-Gel Blue affinity gel is a beaded, cross-linked agarose gel with covalently attached Cibacron Blue F3GA dye. It has a capacity for albumin binding of greater than 11 mg/ml. Affi-Gel Blue gel has been utilized to separate and purify a number of different serum and plasma proteins (Gianazza and Arnaud 1982, Herman and Roberts 1980) and has been used as a first step in the purification of serum proteins by removing the major serum constituent, albumin (Burgett and Greenley 1977). The major advantage of Affi-Gel Blue in the current application is its high albumin capacity. This product has been used to rapidly remove albumin from multiple human serum samples prior to 2-D analysis (Rengarajan et al. 1996). The disadvantage of this ligand is its possible lack of specificity, based on its numerous applications. Although the binding of serum proteins other than albumin and IgG cannot be excluded, the current product has been developed to minimize nonspecific adsorption. Binding conditions have been optimized to saturate the matrix binding sites with the very high-affinity albumin molecules.

Protein A, from *Staphylococcus aureus*, has the property of binding with high specificity the Fc region of IgG molecules (Kronvall and Williams 1969). When coupled to agarose beads, protein A has been extensively used to bind and purify IgG species from various mammalian species (Lindmark et al. 1983). Affi-Gel protein A has a binding capacity of greater than 15 mg human IgG/ml gel.

Section 2

Kit Components

The Aurum serum protein mini kit contains the following components:

Aurum serum protein columns (filled and capped)	10
12 x 75 mm plastic test tubes	10
2.0 ml collection tubes with caps	30
Yellow column tips	10
Aurum serum protein binding buffer	50 ml
Instruction manual	1
Protocol overview	1

Section 3

Storage Conditions

Solutions and columns should be stored at 4°C. Do not freeze. Shelf life is 12 months at 4°C.

Section 4

Necessary Supplies

- Microcentrifuge ($\geq 10,000 \times g$)



2.0 ml collection tube



Aurum serum protein column



12 x 75 mm test tube



Yellow column tip

Fig. 1. Aurum serum protein mini kit

Section 5

Guidelines for Using the Aurum Serum Protein Mini Kit

- Serum samples should be clarified before application to Aurum serum protein columns.
- High salt concentrations (>200 mM) should be avoided due to interference with albumin removal. Salt can also interfere with subsequent IEF analysis. High salt samples can be dialyzed against a low-salt buffer (25 mM phosphate, pH 7.0).
- For serum samples containing a high concentration of albumin, reduce the sample load on the resin from 200 μ l to 150 μ l. The load may need to be further optimized.
- For serum samples with low concentrations of albumin, increase the sample load from 200 μ l to 250 μ l. An alternative would be to dilute the initial sample 1/3 with binding buffer and apply 200–250 μ l. The load may need to be further optimized.
- The bound albumin and IgG can be recovered from the Aurum serum protein columns and analyzed. For 1-D analysis, elute the column with 500 μ l of Laemmli sample buffer (Bio-Rad catalog #161-0737). For 2-D analysis, elute the column with 500 μ l of ReadyPrep™ sequential extraction reagent 3 (Bio-Rad catalog #163-2104).
- If necessary the albumin/IgG-depleted samples can be concentrated using a SpeedVac or lyophilizer.
- Albumin/IgG depleted serum samples, if not analyzed immediately, should be stored at -20°C.
- For 2-D electrophoresis analysis, see bulletin 2561, 2-D Electrophoresis for Proteomics. A Methods and Product Manual.

Section 6

Protocol

Please read Section 5, “Guidelines for Aurum Serum Protein Mini Kit” before proceeding.

1. Place an Aurum serum protein column in a 12 x 75 mm test tube and allow the resin to settle for at least 5 min.
2. Remove the cap and break off the tip from the bottom of the Aurum serum protein column. Return serum protein column to test tube.
3. Start gravity flow in the column and allow residual buffer to drain from the column (approximately 2 min).
4. Once the residual buffer has drained, wash the column with 2 x 1 ml of Aurum serum protein binding buffer using gravity flow. Allow each wash to pass fully through the column and drain.
5. After the last wash, place the column in an empty 2.0 ml collection tube and centrifuge for 20 sec at 10,000 x g in a microcentrifuge to dry resin bed and frit. **Do not overdry resin bed and frit.** Discard the collection tube.
6. Place a yellow column tip on the bottom of the column to stop any residual buffer flow from column. Place the column in a clean 2.0 ml collection tube labeled “unbound”.
7. In a separate 2.0 ml collection tube, prepare sample to be purified by diluting 60 μ l of plasma or serum with 180 μ l of Aurum serum protein binding buffer. Mix by inverting tube several times.
8. Add 200 μ l of the diluted serum sample to the top of the resin bed. Allow sample to penetrate column matrix.
9. Gently vortex the column. Repeat at 5 and 10 min. Allow column to sit an additional 5 min for a total incubation time of 15 min.
10. Remove yellow tip from column and return to 2.0 ml collection tube.

11. Centrifuge the column for 20 sec at 10,000 x g in a microcentrifuge, collecting the eluate in the “unbound” 2.0 ml collection tube.
12. Remove the column and tube together from the centrifuge and wash the resin with 200 μ l of the binding buffer. Replace tube and column in centrifuge.
13. Centrifuge column for 20 sec at 10,000 x g in a microcentrifuge, collecting the eluate in the same “unbound” tube, which contains the albumin- and IgG-depleted serum sample.
14. The treated sample is ready for IEF analysis. For serum, this procedure typically yields protein concentrations around 1.5–2.0 mg/ml as determined by the modified Lowry method (Bio-Rad *DC* protein assay, catalog #500-0112).

Aurum™ Serum Protein Mini Kit

Protocol Overview

Column Setup

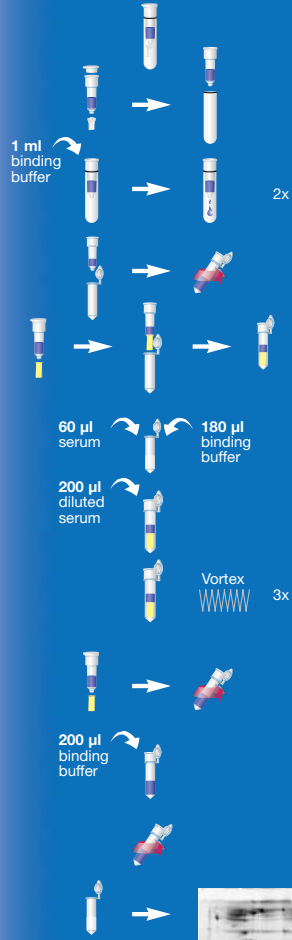
1. Place serum protein column in a test tube for 5 min to allow resin to settle.
2. Remove cap and break tip from column and return to test tube to start gravity flow in column.
3. Wash column with 1 ml of serum protein binding buffer using gravity flow. Repeat.
4. Place column in empty 2.0 ml collection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



BIO-RAD

Fig. 2. Aurum serum protein mini kit protocol overview

Section 7

Troubleshooting Guide

Problem	Possible Cause	Possible Solution
Low protein concentration	Low levels of protein or albumin in sample	Increase serum load on column Concentrate treated sample in SpeedVac
High protein concentration/ insufficient albumin removal	High level of albumin in sample	Decrease the serum load applied to column

Section 8

References

Burgett MW and Greenley LV, Cibacron Blue F3GA affinity chromatography, Amer Lab 9, 74-85 (1977)

Gianazza E and Arnaud P, Chromatography of plasma proteins on immobilized Cibacron Blue F3-GA. Mechanism of the molecular interaction, Biochem J 203, 637-641 (1982)

Herman CA and Roberts R, Purification and immunological characterization of human myocardial MB creatine kinase, Anal Biochem 106, 244-252 (1980)

Kronvall G and Williams RC Jr, Differences in anti-protein A activity among IgG subgroups, J Immunol 103, 828-833 (1969)

Lindmark R et al., Binding of immunoglobulins to protein A and immunoglobulin levels in mammalian sera, J Immunol Methods 62, 1-13 (1983)

Rengarajan K et al., Removal of albumin from multiple human serum samples, BioTechniques 20, 30-32 (1996)

* Cibacron is a trademark of CIBA-Geigy Corp.

Section 9

Ordering Information

Catalog #	Description
732-6713	Aurum Serum Protein Mini Kit, 2 pk , includes 2 serum protein columns, 2 clear 12 x 75 mm polystyrene tubes, 6 sample collection tubes, 10 column tips, 15 ml binding buffer, protocol overview, instructions
732-6701	Aurum Serum Protein Mini Kit, 10 pk , 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

Bio-Rad Laboratories, Inc.

2000 Alfred Nobel Dr.

Hercules, CA 94547 USA

(510) 741-1000

1-800-424-6723