Aurum™ Affi-Gel® Blue Mini Kits and Columns
Catalog #732-6712, 732-6708, 732-6709

Introduction

Aurum Affi-Gel Blue columns consist of Micro Bio-Spin™ columns filled with 100–200 mesh Affi-Gel Blue affinity support. This support allows removal of albumin from serum or plasma samples prior to two-dimensional (2-D) polyacrylamide gel electrophoresis (PAGE) or further fractionation on columns such as Aurum ion exchange mini columns.

Proteins in serum and other biological fluids are difficult to resolve by 2-D PAGE, largely due to the abundance of serum albumin, which constitutes 50–70% of the total protein in human serum. The presence of albumin obscures other proteins in the gel and limits the amount of protein in the serum that can be resolved by 2-D analysis. Furthermore, albumin has wide pI and molecular weight ranges, further reducing resolution and masking many proteins of potential interest. Theoretically, removing 90% of the albumin 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 support is a beaded, crosslinked agarose gel with covalently attached Cibacron Blue F3GA dye. It has been utilized to separate and purify a number of different serum and plasma proteins (Gianazza and Arnaud 1982, Herman and Roberts 1980). 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 cannot be excluded, the current product has been developed to minimize nonspecific adsorption.

Product Description

Matrix	Bio-Gel® A-5m agarose gel
Particle size	80–150 µm (100–200 mesh)
Functional group	Cibacron Blue F3GA
Gel bed volume	0.45 ml
Shipping medium	0.05% NaN_3
Serum capacity	0.1 ml per column
Typical albumin capacity	5 mg per column
Shelf life	1 year at 4°C

Guidelines for Aurum Affi-Gel Blue Columns

- Serum samples should be clarified before application to Affi-Gel Blue 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 application buffer.

  Examples of low-salt application buffers
  20 mM acetate, pH 5.0
  20 mM phosphate, pH 7.0
  20 mM Tris, pH 8.3

- For serum samples containing an extremely high or low concentration of albumin, reduce or increase the sample load according to the capacity data in the Product Description section. Sample diluted with the application buffer can be added to the Affi-Gel Blue column in aliquots if the total sample volume is >0.5 ml.
- The bound albumin can be recovered from Affi-Gel Blue columns and analyzed. For 1-D analysis, elute the column with 500 µl of Laemmli sample buffer (catalog #161-0737). For 2-D analysis, elute the column with 500 µl of ReadyPrep™ sequential extraction reagent 3 (catalog #163-2104).
- If necessary, the albumin-depleted serum samples can be concentrated using a SpeedVac or lyophilizer.
- Albumin-depleted serum samples, if not analyzed immediately, should be stored at –20ºC.
- For 2-D electrophoresis analysis, see bulletin 2651, 2-D electrophoresis for proteomics. A methods and product manual.

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Protocol

1. Place an Aurum Affi-Gel Blue column in a 12 x 75 mm test tube (catalog #732-6714) 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 Affi-Gel Blue column. Return the column to the test tube.
3. Start gravity flow in the column and allow the 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 a low-salt application buffer (refer to the Guidelines for Aurum Affi-Gel Blue Columns section) using gravity flow. Allow each wash to fully drain from the column.
5. After the last wash, place the column in an empty 2.0 ml collection tube (catalog #223-9430) 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 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 125 µl of plasma or serum with 375 µl of the low-salt application buffer. Mix by inverting the capped tube several times.
8. Add 300–400 µl of the diluted serum sample to the top of the resin bed. Allow sample to gravity flow through the column to the "unbound" collection tube.
9. Centrifuge the column for 20 sec at 10,000 x g in a microcentrifuge, collecting the residual eluate in the "unbound" collection tube.
10. Remove the column and tube together from the centrifuge and wash the resin with 400 µl of the low-salt application buffer. Replace tube and column in centrifuge.
11. 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-depleted serum sample.
12. The treated sample is ready for application to another column or IEF analysis. For serum, this procedure typically yields protein concentrations around 3–4 mg/ml as determined by the *DC* protein assay (catalog #500-0112). See bulletin 1069, Colorimetric protein assays.

References

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)

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

2-D electrophoresis for proteomics. A methods and product manual, Bio-Rad bulletin 2651

Colorimetric protein assays, Bio-Rad bulletin 1069

Ordering Information

Catalog #	Description
Aurum Affi-Gel Blue Mini Kits and Columns	
732-6712	Aurum Affi-Gel Blue Mini Kit, 2 pk
732-6708	Aurum Affi-Gel Blue Mini Columns, 25 pk
732-6709	Aurum Affi-Gel Blue Mini Columns, 100 pk
Aurum Ion Exchange Mini Kits and Columns	
732-6710	Aurum AEX Mini Kit, 2 pk
732-6705	Aurum AEX Mini Kit, 10 pk
732-6706	Aurum AEX Mini Columns, 25 pk
732-6707	Aurum AEX Mini Columns, 100 pk
732-6711	Aurum CEX Mini Kit, 2 pk
732-6702	Aurum CEX Mini Kit, 10 pk
732-6703	Aurum CEX Mini Columns, 25 pk
732-6704	Aurum CEX Mini Columns, 100 pk
Aurum Serum Protein Mini Kits	
732-6713	Aurum Serum Protein Mini Kit, 2 pk
732-6701	Aurum Serum Protein Mini Kit, 10 pk
Prepacked Micro Bio-Spin Columns	
732-6221	Micro Bio-Spin 6 Columns, Tris, 25 pk
732-6222	Micro Bio-Spin 6 Columns, Tris, 100 pk
Accessories	
223-9430	EZ Micro™ Test Tubes, 2 ml, natural, 500
732-6714	Test Tubes, 12 x 75 mm, 10
Prepacked Econo-Pac® Cartridges	
732-0101	Econo-Pac Blue Cartridge, 1 x 5 ml
732-105	Econo-Pac Blue Cartridge, 5 x 5 ml
Bulk Media	
153-7301	Affi-Gel Blue Gel, 50–100 mesh, 100 ml
153-7302	Affi-Gel Blue Gel, 100–200 mesh, 100 ml

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