CM Affi-Gel® Blue Gel

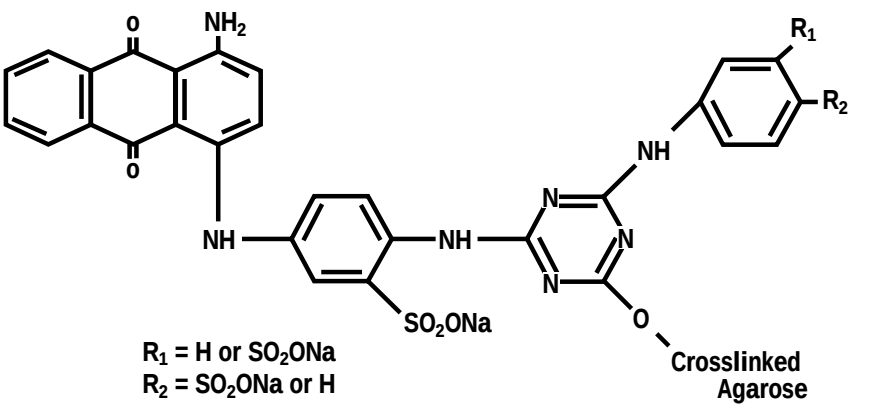
Instruction Manual

Catalog Number
153-7304

BIO-RAD

Introduction

CM Affi-Gel blue gel is a bifunctional affinity/ion exchange chromatography matrix prepared by coupling Cibacron blue F3GA and carboxymethyl (CM) groups to Bio-Gel® A-5m crosslinked agarose gel. The Cibacron blue functions as an ionic, hydrophobic, or sterically active binding site for proteins with dinucleotide folds, such as albumin.



Cibacron blue coupled to agarose.

The carboxymethyl functional group functions as a cation exchanger and will bind proteins with isoelectric points lower than the pH of the mobile phase. (At pHs below 4.5 the carboxymethyl group will be protonated and will not function as an ion exchanger.) This bifunctionality allows CM Affi-Gel blue gel to bind both albumin and protease from serum. Dialysis of

serum is not necessary prior to chromatography on this gel. Albumin and serum protease are bound strongly enough that a high concentration of salt or chaotropic reagent is required to elute these components. Chromatography on CM Affi-Gel blue gel provides a convenient initial step in the purification of serum proteins.

Product Description

Matrix	Bio Gel A-5m agarose gel
Particle size	150–300 µm (50-100 mesh)
Shipping medium	50 mM KCl with 0.04% NaN_3
Functional groups	Cibacron blue and carboxymethyl
Flow rate	15–25 cm/hr
Pressure limit	15 psi
Capacity	
Serum	2 to 6 ml of gel/ml of serum (lot dependent)
Stability	
pH	2–11
Organic solvents	alcohols
Temperature	autoclavable
Storage	1 year at 4 °C, in 0.02% NaN_3 or other preservative

Material required but not supplied

Pre-wash buffer	0.1 M acetic acid, pH 3, 1.4 M NaCl, 40% isopropanol
Running buffer	10 mM K_2HPO_4, pH 7.25, 0.15 M NaCl, 0.02% NaN_3,
Regeneration buffer	2 M guanidine HCl or 1.5 M NaSCN
Buchner funnel	
Chromatography column	

General Instructions

1. Prepare CM Affi-Gel blue gel by washing it on a Buchner funnel, or in a column, with 5 bed volumes of pre-wash buffer, followed by 7 bed volumes of deionized water. This is required the first time the gel is used, or if the gel has been stored for more than 1 week since the previous use. Small amounts of the blue dye may appear in the alcohol wash.* After prewash, equilibrate the column with 3 bed volumes of running buffer.

   The capacity of the gel is lot dependent, and will range between 2–6 ml of gel per ml of serum. The capacity for human and rabbit serum for a specific lot of gel is printed on the label on the bottle.**

2. To insure that the prewash removed excess dye in the gel, wash the gel with 2 bed volumes of 1.4 M NaCl and then with running buffer. If the high salt wash is colored, repeat the first pre-wash.
3. Pack the gel into a suitable column.
4. Apply the serum sample.
5. Wash the column with 2 bed volumes of running buffer at a flow rate of 15–30 cm/hr. The effluent from this step contains the serum proteins minus plasminogen and albumin. Approximately 90% of the globulin will elute in one column bed volume.
6. The albumin can be eluted with 2 bed volumes 1.4 M NaCl in running buffer. This is optional, and can be eliminated.
7. Whether the albumin is eluted or not, regenerate the column with 2 bed volumes of regeneration buffer followed by 2 bed volumes of running buffer.

Note: The first one or two cycles of the gel may show low levels of eluted dye in the high salt peak. This does not affect the functionality of the gel.

8. Some loss of capacity due to small amounts of protein which remain bound to the gel may be evident after about five cycles. To compensate for this, increase the gel-to-sample ratio by 20% for subsequent cycles. The useful life of the gel is generally eight to ten cycles.

Protease Free Globulin Fraction from Serum with CM Affi-Gel Blue Gel

A partially purified globulin fraction is often desirable in the preparation of immunological reagents. This is commonly achieved by a two-step procedure involving fractionation on DEAE anion exchange chromatography followed by ammonium sulfate precipitation.[1] Recovery of active antibody by this method is generally about 65%, and detectable protease activity remains. In fact, ammonium sulfate may serve to activate serum proteases.[2] Better recoveries (80–90%) and complete removal of protease are achieved by using CM Affi-Gel blue affinity gel instead of DEAE cellulose, followed by an ammonium sulfate precipitation as an additional purification and concentration step to obtain a globulin fraction free of protease and serum complement proteins. This procedure is particularly useful when processing large volumes of serum or when high yields (>90%) are desired.

Instructions for a Protease Free Globulin Fraction From Serum

1. Follow the general instructions through step 5.
2. Measure the total volume of the collected protein peak. Determine the amount of solid ammonium sulfate required to achieve a 45% saturated solution.*** While stirring the pooled protein with a stir bar on a stir plate, slowly add the ammonium sulfate to the solution.
3. After all the $(NH_4)_2SO_4$ has been added, continue stirring for at least 1 hour at room temperature. If stirred or stored overnight before centrifugation, the suspension should be kept at 4 °C.
4. Centrifuge the suspension in a refrigerated (4 °C) centrifuge at approximately 1,000 g for 20 minutes. (If supernatant does not appear clear, centrifuge longer.) Discard supernatant.
5. Resuspend the pellet in 45% saturated ammonium sulfate by breaking up pellet with a pipet and centrifuge again.
6. Resuspend pellets in a minimum volume of running buffer. Do not mix vigorously to dissolve pellets (this will denature proteins). If pellet does not go into solution readily, let sit in PBS at 4 °C for 1–2 hours, and then mix.

7. Remove any remaining ammonium sulfate, by desalting on Bio-Gel P-6DG desalting gel or prepacked desalting columns using gravity flow, with the Econo-Pac® P6 cartridge, or by dialysis.
8. Continue with step 6 of the general instructions for CM Affi-Gel blue gel column regeneration.

References

1. Hudson, L. and Hay, F. C., *Practical Immunology*, p. 152, Blackwell Scientific Publications, Oxford, England (1976).
2. Steinbuch, M., Audran, R. and Pejaudier, L., *C. R. Soc. Biol.*, **164,** 296 (1970).
3. Gee, A. P., Borsos, T. and Boyle, M. D. P., *J. Immunol. Methods*, **30,** 119 (1979).
4. Kelleher, P C., Smith, C. J. and Parnell, R., *J. Chromatog.*, **173,** 415 (1979).
5. Gianazza, E. and Arnaud, P., *Biochem. J.*, **201,** 129 (1982).
6. Ledden, D. J., Feldhoff, R. C. and Chan, S. K., *Biochem. J.*, **205,** 331 (1982).

* Note: If this alcohol wash is done in a column you may notice shrinkage of the gel.

** For species other than human or rabbit, use the higher gel/serum level indicated on the CM Affi-Gel blue gel label.

*** 275 grams $(NH_4)_2SO_4$ per liter.

Ordering Information

Catalog Number	Product Description
153-7304	**CM Affi-Gel Blue Gel,** 100 ml
	For desalting and sample preparation:
150-0738	**Bio-Gel P-6DG Desalting Gel,** 100 g
150-0739	**Bio-Gel P-6DG Desalting Gel,** 1 kg
732-2010	**Econo-Pac 10DG Desalting Columns,** 10 ml, 30
732-0011	**Econo-Pac P6 Cartridge,** 5 ml

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