DEAE Affi-Gel® Blue Gel

Instruction Manual

Catalog Number
153-7307

BIO-RAD

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4006049 Rev A

Introduction

DEAE Affi-Gel Blue gel is a bifunctional affinity/ion exchange chromatography matrix prepared by coupling Cibacron® blue F3GA and diethylaminoethyl groups to Bio-Gel® A-5m, agarose gel. The Cibacron blue F3GA functions as an ionic, hydrophobic, or sterically active binding site for proteins with dinucleotide folds, such as albumin. The diethylaminoethyl functional group functions as an anion exchanger and will bind proteins with isoelectric points higher than the pH of the mobile phase. This bifunctionality makes DEAE Affi-Gel Blue gel a very powerful tool. It is possible to obtain highly purified IgG from serum from a wide variety of species through carefully optimizing the ionic strength and pH of the application buffer.

Chromatography on DEAE Affi-Gel Blue gel provides a convenient initial step in the purification of serum proteins. After the IgG has eluted, additional fractions can be obtained from the DEAE Affi-Gel Blue gel by elution with an ionic strength gradient. DEAE Affi-Gel Blue gel has a greater binding affinity for serum albumin than DEAE ion exchangers, and offers a

superior method of obtaining serum fractions uncontaminated by albumin.

Product Description

Matrix	Bio Gel A-5m agarose gel
Particle size	150-300 µm (50-100 mesh)
Shipping medium	0.01M Tris, pH 8, 0.15 M NaCl, 0.04% NaN_3
Functional Groups	Cibacron blue and diethylaminoethyl
Typical flow rate*	15-25 cm/hr
Pressure limit	15 psi
Capacity**	
Serum	0.2-1 ml of serum/ml of gel
Recovery of IgG	>55%
Recovery of Albumin	>90%
Removal of protease	100%
Stability	
pH	2-11
Organic solvents	alcohols
Temperature	not autoclavable
Storage	1 year at 4 °C, in 0.02% NaN_3 or other preservative

*Flow rate determined using a 1.5 x 20 cm column, and a hydrostatic pressure of 1:1.

**The capacity for rabbit serum and human serum is lot to lot dependent. It is determined on every lot, and provided on the label.

Material Required but not Supplied

Pre-wash buffer	0.1 M acetic acid, pH 3, 1.4 M NaCl, 40% isopropanol
Running buffer	see Table 2
Regeneration buffer	2 M guanidine HCl in application buffer, or 1.5 M NaSCN
Buchner funnel	
Chromatography column	

General Instructions

1. Prepare the appropriate buffer using the information given in Table 1. Accurate buffer preparation is essential for optimum IgG recovery. See Optimizing the Separation Conditions.

Table 1. Buffers for Purification of IgG from Serum on DEAE Affi-Gel Blue gel

Species	Application Buffer
Rabbit	20 mM Tris-HCl, pH 8.0, 25 mM NaCl, 0.02% NaN_3
Sheep	as for rabbit
Goat	as for rabbit
Human*	20 mM K_2HPO_4, pH 8.0, 0.02% NaN_3
Mouse	20 mM Tris-HCl, pH 7.2, 25-50 mM NaCl

These buffers are recommended starting points. Minor changes may be needed. See Optimizing the Separation Conditions.

*KH_2PO_4 is used to prepare this buffer. The pH is adjusted with KOH.

2. Transfer the serum sample to the buffer. This should be done using Bio-Gel P-6DG for buffer exchange, or through dialysis.
3. Rinse the gel on a Buchner funnel with a least 5 bed volumes of pre-wash buffer. DEAE Affi-Gel Blue gel has an excess of dye. This wash will elute residual dye which might be eluted in serum protein fractions. Continue rinsing with at least 10 bed volumes of application buffer (Table 1) to insure that the ionic strength is lowered. Note: If the alcohol wash is done in a column, you may notice shrinkage of the gel.
4. Prepare a column of DEAE Affi-Gel Blue gel using the gel-to-serum volume ratio given on the bottle label. Calculate the total volume of gel needed using the initial serum volume, prior to buffer exchange or dialysis.

   The capacity of the gel is lot dependent, and will range between 0.2 and 1 ml of serum per ml of gel. The capacity for human and rabbit serum for a specific lot of gel is printed on the label of the gel bottle. For species other than human or rabbit, use the lower capacity given.

5. Elute the column with three volumes of application buffer.
6. Apply the serum sample and elute with three bed volumes of starting buffer. Collect fractions which are approximately the volume of the applied sample.
7. Beginning with the first tube of unbound protein peak, combine effluent tubes to give a total volume equivalent to eight times the volume of the initial serum sample.
8. For the isolation of other serum proteins, the column may be eluted with a gradient of increasing salt concentration. A final NaCl concentration of 0.5 M is usually enough to elute all serum proteins except albumin.
9. Most of the bound albumin can be eluted by washing the column with 2 to 3 bed volumes of 1.4 NaCl in application buffer, or with regeneration buffer.

10. Regardless of whether the albumin is eluted or not, regenerate the column with 4 to 5 bed volumes of regeneration buffer followed by 3 bed volumes of application buffer.
11. 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. For serum preparations, the useful life of the gel is generally eight to ten cycles.

Note: The first one or two runs using the gel may show low levels of eluted dye in the high salt peak. This dye does not interfere with the usefulness of the gel and should not appear in subsequent runs. Leaching of the dye can be avoided by washing the column with the prewash buffer.

Optimizing the Separation Conditions

The end result is highly dependent on the pH and ionic strength of the sample. It is crucial that serum is transferred into the appropriate application buffer, either

through desalting using the Bio-Gel P-6DG gel, or through dialysis. The buffers listed in Table 1 have provided complete plasminogen removal when tested, but some variation in non-IgG protein binding and IgG recovery may be encountered using different serum preparations. For this reason, the unbound protein fraction, ideally containing only IgG, should always be tested for proteolytic activity as described above. If a sample displays minor amounts of proteolytic activity in the unbound protein fraction, it may be necessary to modify the buffer. This experiment may also help to determine conditions for even higher recoveries of IgG in the unbound fraction.

Transfer a sample to 20 mM Tris-HCl, pH 8.0, and apply it to a column equilibrated with the same buffer. Elute the column with a sodium chloride gradient, and collect fractions of the same volume as the sample applied. The sodium chloride concentration at which plasminogen elutes can be determined by testing the effluent for proteases. Fractions free of protease can be pooled, or a second serum sample can then be purified at a sodium chloride concentration slightly lower than that at which non-IgG protein is detected. For higher purity or

improved recovery of IgG, use Affi-Gel Protein A support.

For more information and applications, request Bulletin 1092.

Catalog Number	Product Description
153-7307	**DEAE Affi-Gel Blue Gel**
	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,** 30 x 10 ml
732-0011	**Econo-Pac P-6 Cartridge,** 5 ml
	Other products for IgG purification
732-0031	**Econo-Pac DEAE Cartridge,** 1 x 5 ml
732-0035	**Econo-Pac DEAE Cartridge,** 5 x 5 ml
732-2026	**Econo-Pac Serum IgG Purification Columns**
732-2027	**Econo-Pac Serum IgG Purification Kit**