Affi-Gel® Blue Gel

Instruction Manual

Catalog Numbers
153-7301
153-7302

BIO-RAD

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Section 1
Introduction

Affi-Gel blue affinity gel is a beaded, crosslinked agarose gel with covalently attached Cibacron® Blue F3GA dye. It contains ≥1.9 mg dye per ml of gel, and has a capacity for albumin binding of greater than 11 mg/ml. Affi-Gel blue gel purifies a large range of proteins from widely divergent origins. The blue dye functions as an ionic, hydrophobic, aromatic, or sterically active binding site in various applications. Proteins that interact with Affi-Gel blue gel can be bound or released with a high degree of specificity by manipulating the composition of the eluant buffers. In many cases, one can also predict what will interact with the matrix and the general conditions under which binding and elution will occur.

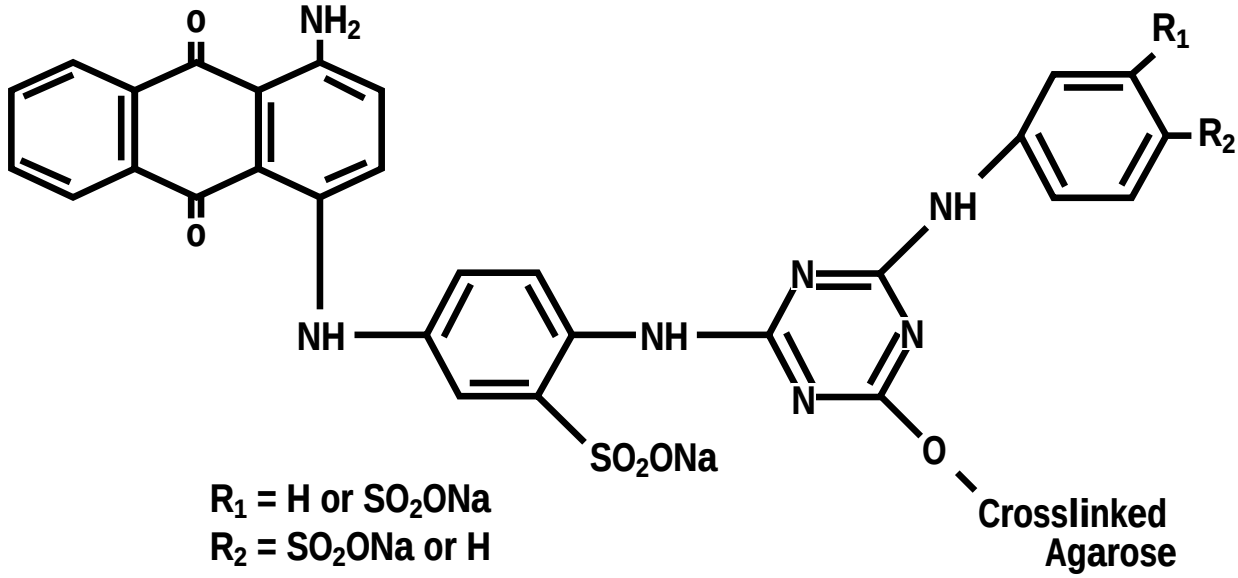

Fig. 1. Cibacron blue coupled to agarose.

Affi-Gel blue gel is supplied ready to use as an aqueous slurry of fully hydrated gel. It is available in two convenient particle sizes: a faster flowing 50-100 mesh (150-300 µm) and a slower flowing, higher capacity 100-200 mesh (75-150 µm). The gel is also available in convenient Econo-Pac® cartridges which can be used with the Econo System, FPLC®, and HPLC systems.

Section 2
Product Description

Matrix	Bio-Gel A-5m agarose gel
Particle Sizes	150-300 µm (50-100 mesh) 80-150 µm (100-200 mesh)
Shipping Medium	0.05% NaN_3
Functional Group	Cibacron blue
Typical Flow Rate*	15-25 cm/hr
Pressure limit	15 psi
Serum Capacity	0.2 ml/ml gel
Typical Albumin Capacity	11 mg/ml
Stability	
pH	4-10
Organic Solvents	alcohols
Temperature	4-30 °C
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

Section 3
Instructions for Use

3.1 Sample Preparation

Proper adjustment of the sample pH and ionic strength is critical for consistent and reproducible results when using dye affinity gels. The sample must be exchanged into the appropriate application buffer. This can be achieved by exchanging it into the application buffer using Econo-Pac 10DG desalting columns, Bio-Gel P-6DG desalting gel, or the Econo-Pac P6 cartridge. The choice of product depends on the sample volume. Alternatively, the sample can be dialyzed against the application buffer. All samples should be filtered through a 0.45 µm filter.

3.2 Albumin Removal

Affi-Gel blue gel provides a simple first step in the purification of many serum proteins by removing the major serum constituent, albumin. The binding of albumin

is so strong that a high concentration of salt or chaotropic reagent is required to desorb the albumin. Other serum proteins either do not bind to Affi-Gel blue gel or can be eluted with relatively low concentrations of salt.

Table 1. Buffer Formulations For Albumin Removal Procedure

A Buffer	20 mM phosphate buffer, pH 7.1
B Buffer	1.4 M NaCl, in 20 mM phosphate buffer, pH 7.1
C Buffer	2 M guanidine HCl in 20 mM phosphate buffer, pH 7.1
or	1.5 M NaSCN in 20 mM phosphate buffer, pH 7.1

Procedure

1. Prepare a column of Affi-Gel blue gel, 50-100 mesh, with a total bed volume of 5 ml of gel per milliliter of serum to be processed.
2. Prewash the column with 2 bed volumes of buffer A.

3. Equilibrate the serum sample in buffer A by dialyzing overnight, or by rapid column desalting in a column of Bio-Gel P-6 DG gel, on an Econo-Pac 10DG column, or on an Econo-Pac P6 cartridge.
4. Apply the equilibrated serum sample to the column.
5. Wash the column with 2 bed volumes of buffer A. The effluent from this step contains the serum proteins minus most of the albumin.
6. Optional step: elute the albumin with buffer B.
7. Whether or not the albumin was eluted, regenerate the column with 2 bed volumes of buffer C.

3.3 Enzyme Purification

Affi-Gel blue gel has been used to purify a number of enzymes. It has been particularly useful in the purification of kinases, dehydrogenases, and other nucleotide-dependent enzymes. The degree of purification obtained with Affi-Gel blue gel is typically much greater than that obtained using biospecific affinity chromatography. It has been suggested that enzymes containing a “dinucleotide fold” bind

biospecifically to the Cibacron Blue F3GA dye.[1] In many cases, the enzyme can be eluted from the Affi-Gel blue gel with a specific nucleotide cofactor. Often, salt is more effective for elution, indicating that other mechanisms are sometimes involved.[2]

Table 2 lists some applications in which Affi-Gel blue gel has been used for enzyme purification.

Table 2. Affi-Gel Blue Gel Enzyme Applications

Enzyme	Source
1-aminocyclopropane-1-carboxylate synthase	Tomato pericarp tissue
3',5' cyclic AMP phosphodiesterase	Dictyostelium discoideum
3'-PGA diesterase	Yeast
5-methyl-L-tetrahydrofolate reductase	Bovine liver
Acylation stimulating protein	Human plasma
Adenylate cyclase	Bovine brain
Alkaline phosphatase	*E. coli*
Alkyl hydroperoxide reductase	*S. thphimurium*
ATP:AMP phosphotransferase	Bovine heart
Calmodulin-dependent cyclic nucleotide phosphodiesterase	Bovine brain
Carbamyl phosphate synthetase	Frog liver
DNA polymerase	Calf thymus
Formamidopyrimidine-DNA glycosylase	*E. coli*
Glutamate dehydrohenase	Yeast

Eluant	Reference
50 mM KPO_4, 5 µM pyridoxal phosphate	41
0.18 M NaCl	3
0.6 M KCl in 50 mM Tris-HCl	35
0.4-3.0 M KCl	6
3 M NaCl, 0.02 M phosphate	37
1 mM KCl, 8 mM ATP, 16 mM $MgCl_2$, 1 mM EDTA	24
-	39
1.0 M KCl, 0.5 M NaCl	36
0.4-2.0 M NaCl	10
0.15-1.5 M NaCl	23
1 mM dithiothreitol	14
0-0.5 M KCl	12
0.1-0.8 M KCl	4
10 mM NADH, 1 M NH4Cl	15

Table 2. Affi-Gel Blue Gel Enzyme Applications *(continued)*

Enzyme	Source
Glutamine synthetase	*Salmonella typhimurium*
Glyoxalase II	Rat erythrocytes
GMP reductase	Human erythrocytes
GTP:RNA guanylyltransferase	Wheat germ
Isocitrate dehydrogenase	*E. coli*
Isocitrate dehydrogenase	*E. coli*
MB creatine kinase	Human heart
Membrane-bound phosphatidylinositol kinase	Rat brain
Methylenetetrahydrofolate reductase	Porcine liver
Metmyoglobin reductase	Bovine heart
Phosphodiesterase	Bovine brain
Replication protein A (RP-A)	Animal cells
RNA ligase	*E. coli*
Serine transhydroxymethylase	Porcine liver
Thymidylate	*Saccharomyces cervisiae*
Tyrosine phenollyase	*Erwinia herbicola*

Eluant	Reference
5 mM ATP	8
0-0.2 M KCl	19
1 mM NADPH, 2 mM GMP	21
0.05-0.75 M NaCl	5
2 mM NADP	16
2 mM NADP+	13
0.25 M NaCl	7
Triton X-100, NaCl, glycerol	38
0-10 mM NADPH	17
1.0 M NaCl, 1 mM NADP+	11
0.2 M NaCl	25
-	40
0.2 M NaCl, 2 mM ATP	20
0.5 M KCl	18
0.25-1 M KCl	22
1.5 M NaCl, 0.5 mM mercaptoethanol	9

Suggested Procedure for Enzyme Purification

1. Prepare a column of Affi-Gel blue gel, 100-200 mesh. A 5 ml bed volume for every 20 mg of protein to be chromatographed should be sufficient. Equilibrate the column with starting buffer. The starting buffer should be of low ionic strength, 50 mM or less. (Published methods have used pH values from 6.0 to 8.5)
2. Dialyze sample against starting buffer. Alternatively, the sample can be rapidly desalted in a column of Bio-Gel P-6 DG gel, on an Econo-Pac 10DG column, or on an Econo-Pac P6 cartridge.
3. Apply the sample to the column.
4. Wash the column with 2 bed volumes of starting buffer.
5. Check the effluent for enzyme activity. If it is not bound, then alter conditions. Change the pH, decrease the ionic strength, or change the buffer.
6. The column may be eluted with a salt gradient or with a competitive eluant such as a cofactor. Table 2 contains examples of eluants used in Affi-Gel blue gel chromatography.

7. Regenerate the column by washing it with 2 bed volumes of 2 M guanidine HCl or 1.5 M NaSCN, followed by 2 bed volumes of the starting buffer.

3.4 Purification of Blood Proteins

Affi-Gel blue gel has been used to separate and purify a number of different serum and plasma proteins. Table 3 lists several examples. Gianazza and Arnaud[29,30] have developed a single step method for general fractionation of plasma proteins. By altering pH and ionic strength, twenty-seven plasma proteins were separated, providing better initial purification of individual proteins than many of the two and three step methods commonly used.

Table 3. Purification of Blood Proteins on Affi-Gel Blue Gel

Application	Reference
Purification of human serum complement	26
Purification of rat alpha fetoprotein	27
Purification of rat serum albumin	28
Separation and purification of plasma proteins	29,30
Purification of alpha-2-macroglobulin	31

Section 4
Storage Conditions

The Affi-Gel blue gel should be stored at 4 °C in 0.02% sodium azide.

Section 5
References

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Section 6
Product Information

Catalog Number	Product Description
153-7301	Affi-Gel Blue Gel, 50-100 mesh, 100 ml
153-7302	Affi-Gel Blue Gel, 100-200 mesh, 100 ml
732-0101	Econo-Pac Blue Cartridge, 1 x 5 ml
732-0105	Econo-Pac Blue Cartridge, 5 x 5 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, 1 x 5 ml
732-0015	Econo-Pac P6 Cartridge, 5 x 5 ml

Section 7
Technical Information

For additional information and technical assistance, contact your local Bio-Rad representative or call Technical Service at 1-800-4BIORAD.

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