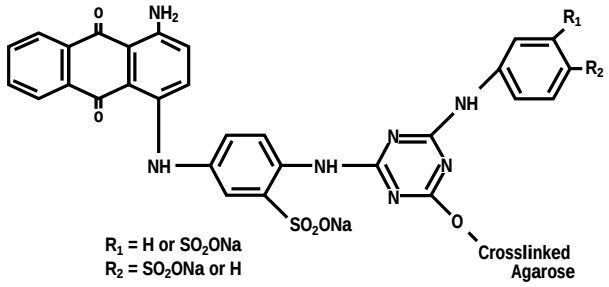
US/EG Bulletin 1107

Affi-Gel® Blue Affinity Chromatography Gel for Enzyme and Blood Protein Purifications

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 typical albumin capacity 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. Structure of Affi-Gel blue gel.



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, 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 HLPC systems.

Specifications

Functional group:	Cibacron blue
Ligand concentration:	≥ 1.9 mg/ml
Typical protein capacity:	> 11 mg/ml
pH stability:	4–11
Pressure limit:	15 psi
Flow rate:	15–25 cm/hr
Bead size:	
50-100 mesh:	150–300 µm
100-200 mesh:	75-150 µm
Storage:	4 °C
Shipping buffer:	0.05 % NaN_3 in water
Sanitization:	0.1 M NaOH

Applications

Purification of Blood Proteins

Affi-Gel blue gel has been used to separate and purify a number of different serum and plasma proteins. Table 1 lists several examples. Gianazza and Arnaud have developed a single step method for general fractionation of plasma proteins.[29,30] 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 1. 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
Characterization of fragments of human albumin	32
Albumin removal from serum	33, 40
Isolation of sarcoplasmic reticulum vesicles	34
Purification of equine thyromedin	45
Affinity separation of human plasma gelsolin	47

Removal of Albumin

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. Affi-Gel blue gel can be regenerated 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.

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.

Technical Assistance

For additional information and technical assistance, contact your local Bio-Rad representative. In the USA and Canada call 1-800-4BIORAD.

Ordering 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
732-1010	**Econo-Pac Columns,** empty, 50

Table 2. Affi-Gel Blue Gel Enzyme Applications

Enzyme	Source	Eluant	Reference
3',5' cyclic AMP phosphodiesterase	*Dictyostelium discoideum*	0.18 M NaCl	3
3'-PGA diesterase	Yeast	0.6 M KCl in 50 mM Tris-HCl	35
5-methyl-L-tetrahydrofolate reductase	Bovine liver	0.4–3.0 M KCl	6
Acylation stimulating protein	Human plasma	3 M NaCl, 0.02 M phosphate	37
Adenylate cyclase	Bovine brain	1 mM KCl, 8 mM ATP, 16 mM $MgCl_2$, 1 mM EDTA	24
Alkaline phosphatase	*E. coli*	—	39
Alkyl hydroperoxide reductase	*S. typhimurium*	1.0 M KCl, 0.5 M NaCl	36
Aspergillins (RIPs)	Aspergillus	0.5–1 M NaCl gradient	41
ATP:AMP phosphotransferase	Bovine heart	0.4–2.0 M NaCl	10
Calmodulin-dependent cyclic nucleotide phosphodiesterase	Bovine brain	0.15–1.5 M NaCl	23
Candida acid proteinase (CAP)	*C. albicans*	1.5 M NaCl in citrate buffer	42
Carbamyl phosphate synthetase	Frog liver	1 mM dithiothreitol	14
Casein kinase I	Bovine sperm	0.25–2.5 M NaCl gradient	43
DNA helicase	*S. cerevisiae*	1 M NaCl	44
DNA polymerase	Calf thymus	0–0.5 M KCl	12
Formamidopyrimidine-DNA glycosylase	*E. coli*	0.1–0.8 M KCl	4
GAL4(63)	*S. cerevisiae*	STD, 250 mM NaCl	46
Glutamate dehydrogenase	Yeast	10 mM NADH, 1 M NH_4Cl	15
Glutamine synthetase	*S. typhimurium*	5 mM ATP	8
Glyoxalase II	Rat erythrocytes	0–0.2 M KCl	19
GMP reductase	Human erythrocytes	1 mM NADPH, 2 mM GMP	21
GTP:RNA guanylytransferase	Wheat germ	0.05–0.75 M NaCl	5
Isocitrate dehydrogenase	*E. coli*	2 mM NADP	16
Isocitrate dehydrogenase	*E. coli*	2 mM $NADP^+$	13
MB creatine kinase	Human heart	0.25 M NaCl	7
Membrane-bound phosphatidylinositol kinase	Rat brain	Triton X-100, NaCl, glycerol	38
Methylenetetrahydrofolate reductase	Porcine liver	0–10 mM NADPH	17
Metmyoglobin reductase	Bovine heart	1.0 M NaCl, 1 mM $NADH^+$	11
Mitochondrial hexokinase	Rat brain	Glucose 6-phosphate	48
Phosphodiesterase	Bovine brain	0.2 M KCl	25
Phosphatidic acid phosphatase	Porcine thymus membranes	0.25–3 M NaCl gradient	49
Phosphoinositiede-specific phospholipase C (PIC)	Bovine heart	2 M NaCl	50
Polyadenylate-binding protein (PABP)	*P. sativum*	2 M Guanidine-HCl	51
RNA ligase	*E. coli*	0.2 M NaCl, 2 mM ATP	20
Serine transhydroxymethylase	Porcine liver	0.5 M KCl	18
Thymidylate synthetase	*S. cervisiae*	0.25–1 M KCl	22
Tyrosine phenollyase	*Erwinia herbicola*	1.5 M NaCl, 0.5 mM mercaptoethanol	9

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