DEAE and CM Bio-Gel® A Ion Exchange Gels

Instruction Manual

Catalog Numbers 153-0740 and 153-0840

BIO-RAD

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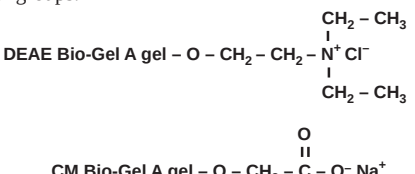
Section 1
Introduction

DEAE and CM Bio-Gel A ion exchangers are based on 4% crosslinked agarose beads. The gels show negligible shrinking or swelling with changes in pH or ionic strength, which permit regeneration of the gel in the column. They are designed for chromatography of proteins, and are notable for their high yields of proteins. The Bio-Gel A ion exchangers are often used to replace older dextran or cellulose based material, with little or no change in the existing protocol. The Bio-Gel A ion exchangers are supplied hydrated, and do not require activation with base or acid as do old cellulose based materials. CM Bio-Gel A gel can also be used in affinity chromatography. EDAC (1-(3-dimethylamionpropyl)-3-ethyl carbodiimide hydrochloride) is used to activate the carboxyl groups and then couple amino terminal ligands, proteins or spacer arms.

Section 2
Technical Description

The DEAE Bio-Gel A gel is a weak anion exchanger with diethylaminoethyl functional groups. The CM Bio-Gel A gel is a weak cation exchanger with carboxymethyl functional groups.



Bio-Gel A ion exchangers exhibit very little swelling or shrinking with changes in pH or ionic strength (see Figure 1A and 1B).

Both ion exchangers have an even capacity within their working pH range (see Figure 2). The macroporous nature of the gel allows both small and large molecules to access the exchange sites located throughout it.

A more detailed description is given in Table 1.

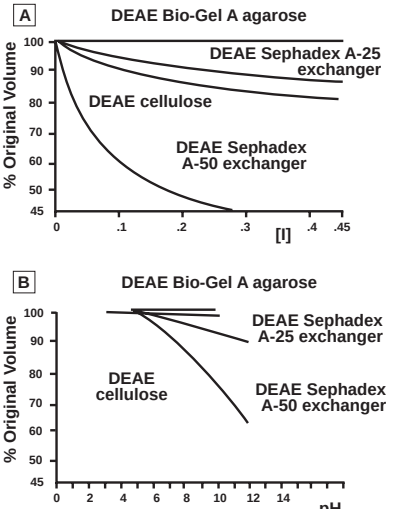


Figure 1A. Changes in bed volume with changes in pH and ionic strength for DEAE Bio-Gel A agarose and corresponding cellulose and dextran exchangers. (A) DEAE exchangers with changes in ionic strength. (B) DEAE exchangers with changes in pH.

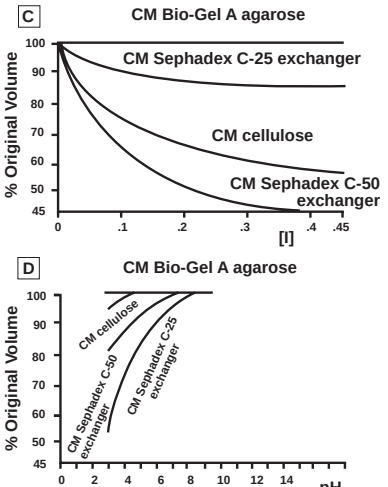


Figure 1B. Changes in bed volume with changes in pH and ionic strength for CM Bio-Gel A agarose and corresponding cellulose and dextran exchangers. (C) CM exchangers with changes in ionic strength. (D) CM exchangers with changes in pH.

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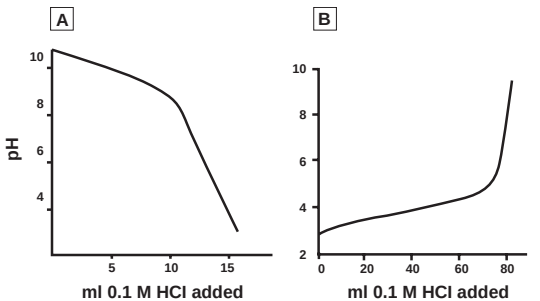

Fig. 2. Titration curves for Bio-Gel A ion exchangers. (A) DEAE Bio-Gel A gel, 100 ml in free base form, in 0.15 M NaCl, titrated with 0.1 M HCl. pH was measured potentiometrically. (B) CM Bio-Gel A gel, 100 ml titrated with 0.1 M NaOH in the presence of 0.5 M NaCl.

Table 1. Bio-Gel A Ion Exchanger Product Description

	DEAE Bio-Gel A	CM Bio-Gel A
Type of exchanger	Weak anion	Weak cation
Functional group	$-ON^{+}(CH_2CH_3)_3$	$-OCH_2COO^{-}$
Base material	Bio-Gel A-5m, medium	
Working pH range	2-9.5	4.5-10
Ionic capacity	20±5 µeq/ml	20±5 µeq/ml
Counterion	Cl^{-}	Na^{+}
Protein binding capacity (hemoglobin, mg)	45±10*	45±10**
Particle size range	75-150 µm	75-300 µm
Exclusion limit	5,000,000	5,000,000
Linear flow rate (1.5 x15 cm column, 2:1 head:bed ratio)	>20 cm/hour	>20 cm/hour
Chemical stability		
in 10 mM HCl	Excellent	Excellent
and 10 mM NaOH	Excellent	Excellent
Volume change		
pH	see Figure 1	see Figure 1
Ionic strength	see Figure 1	see Figure 1
Temperature range	2-30° C	2-30° C
Hydrated, aqeous solution	pH 8	pH 7
Antimicrobial agent	0.05% NaN_3	0.05% NaN_3

* Determined in 5 mM Tris-HCl, pH 8.6
** Determined in 10 mM sodium acetate, pH 5.0

Section 3
Instructions for Use

3.1 Preparation of the Gel

Bio-Gel A ion exchange gels are supplied fully hydrated in a water solution with 0.05% sodium azide added as preservative. The support may be prepared by decanting the excess solution and resuspending the matrix in application buffer prior to column packing (Column Packing, Step 1). It is not necessary to wash the support in base or acid: it is ready to use, as soon as it is packed and equilibrated in starting buffer.

3.2 Column Packing

1. Slurry, approximately 1:1 (v/v), the prepared matrix in the application buffer and degas.
2. Close the outlet of the column. Fill 10% of the column with degassed starting buffer. Remove any air bubbles that might be trapped in the bed support or the column end piece.
3. Add an appropriate amount of the matrix in an even slurry to the column.
4. Fill the remainder of the column with buffer.

5. Connect the flow adaptor to the pump, fill it with buffer, and make sure it is free from air bubbles. Attach the flow adaptor to the column. Inserting it at a slight angle makes it easier to avoid trapping air bubbles.
6. Open the column outlet and pump at least 4-5 bed volumes through the column at a flow rate approximately 20% higher than the flow rate to be used in the application.
7. Switch off the pump and close the column outlet. Remove the inlet tubing from the buffer reservoir, release the pump pressure plate, and adjust the flow adaptor until it is in contact with the gel surface. During this step, buffer will back-flow through the flow adaptor.
8. Re-insert the inlet tubing in the buffer reservoir (remove any air trapped in the tubing, if necessary), tighten the pump pressure plate, open the column outlet, and run 4-5 more column volumes of start buffer, then repeat steps 7 and 8 for a final adjustment of the flow adaptor. Optimal column packing can be achieved with approximately 10 bed volumes of packing buffer.
9. After equilibration in the starting buffer, the column is ready for sample application.

3.3 Sample Preparation

It is advisable to transfer the sample to starting buffer before applying it to the column. This can be done either through buffer exchange using gel filtration on Bio-Gel P-6DG or dialysis.

3.4 Operating Conditions

All buffers commonly used for anion or cation exchange chromatography can be used with the appropriate ion exchange supports (see Table 2). The chemical stability and broad operating pH range of these ion exchangers allow the use of a variety of buffers. It is best to use buffering ions which have the same charge as the functional group on the ion exchanger, e.g. phosphate (-) with a cation exchanger, and Tris (+) with an anion exchanger. The purification may be optimized by changing the ionic strength of the elution buffer, changing the pH, modifying the gradient profile, or experimenting with different buffers. The optimum buffer strength for selective binding is 5-50 mM, and 0.1-0.5 M for elution. Figures 3A and 3B show two elution schemes.

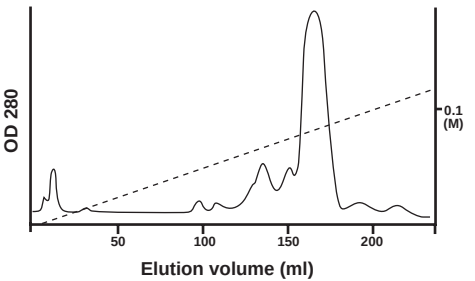

Figure 3A. Chromatography of ribonuclease on CM Bio-Gel A gel. Column: 0.9 x 20 cm. Buffer: 5 mM Tris-HCl, pH 8.0, with NaCl gradient as shown. Sample: 100 mg ribonuclease in buffer.

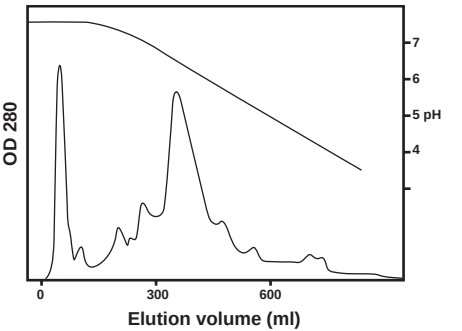

Figure 3B. Separation of fresh pooled human serum. A sample of 8 ml serum was dialyzed against initial buffer. The sample was then applied to a 1.5 x 30 cm of DEAE Bio-Gel A column. Conditions: flow rate, 60 ml/hr; initial buffer, tris succinate 40 mM, pH 8.6; limit buffer, tris succinate 0.3 M, pH 4.3; total gradient, 900 ml. These conditions are those of Himmelhoch and Peterson, *Anal. Biochem.,* **17,** 383 (1966).

Table 2. Common Buffers for Ion Exchange Chromatography[1,2,3]

Type of Ion Exchanger	Buffer	Buffering Range
Cation	Acetic acid	4.8-5.2
	Citric acid	4.2-5.2
	HEPES	7.6-8.2
	Lactic acid	3.6-4.3
	MES	5.5-6.7
	MOPS	6.5-7.9
	Phosphate	6.7-7.6
	PIPES	6.1-7.5
	Pivalic acid	4.7-5.4
	TES	7.2-7.8
	Tricine	7.8-8.9
Anion	Bicine	7.6-9.0
	Bis-Tris	5.8-7.2
	Diethanolamine	8.4-8.8
	Diethylamine	9.5-11.5
	L-Histidine	5.5-6.0
	Imidazole	6.6-7.1
	Pyridine	4.9-5.6
	Tricine	7.4-8.8
	Triethanolamine	7.3-8.0
	Tris	7.5-8.0

Section 4
Regeneration

4.1 Salt Gradients

After each run the gel bed should be washed with 2-4 bed volumes of 0.5-1.0 M NaCl in running buffer to remove reversibly bound material. After re-equilibration in 4-6 bed volumes of starting buffer the next sample can be applied.

4.2 pH Gradients

When using pH gradients, a CM Bio-Gel A column can be regenerated by raising the pH to 11, and allowing 2-4 column volumes run through the column. A DEAE Bio-Gel A column can be regenerated by lowering the pH below 4. Elution with concentrated starting buffer (0.1 M) followed by starting buffer (5-50 mM) completes the regeneration.

4.3 Cleaning and Sanitation

When a column no longer yields reproducible results, the gel may require thorough cleaning and sanitation to remove strongly bound contaminants. The agarose ion exchange supports are most efficiently regenerated in a column with the procedures given below.

1. Wash the gel in the column with 2-4 bed volumes of 10 mM NaOH (DEAE) or 10 mM HCl (CM) at the operating flow rate.
2. Equilibrate with at least 4-5 bed volumes of starting buffer.
3. Check the conductivity and pH of the effluent to verify that the column is equilibrated in the starting buffer before loading the sample.
4. 50% ethylene glycol or ethanol solution can be used to remove hydrophobically bound contaminants.

   Instead of NaOH and HCl you can also use up to 4 M NaCl, 3 M NaSCN, or 6 M urea.

Section 5
Immobilization of Proteins on CM Bio-Gel A Gel

Protein can be immobilized on CM Bio-Gel A gel using the water soluble carbodiimide EDAC. Since the crosslinked matrix contains about 20 μmoles of carboxylate groups per ml of gel, high capacity affinity columns containing 5-15 mg protein per ml of gel can be prepared.

In order to achieve maximum coupling, the pH should be at or below the pI of the protein, and the ionic strength low. This causes the protein to ionically bind to the gel prior to coupling.

Table 3 lists several proteins that have been coupled to CM Bio-Gel A gel in our laboratories.

Table 3. Results of Coupling Various Proteins to CM Bio-Gel A Gel

Coupled Protein	Coupling Buffer*	mg/ml Coupled	% Protein Coupled	Application
Human IgG	A	12	71%	Antibody purification
Human serum albumin	B	12.2	41%	Antibody purification
Soybean trypsin inhibitor	B	17	93%	Removal of serum protease
Human colostrum IgA	C	4.54	56%	Antibody purification

* Buffers A, B, C are explained under Immobilization Procedure.

5.1 Immobilization Procedure

Coupling Buffers

A. 3 mM Phosphate buffer, pH 6.3

B. 1 mM Pyridine/HCl buffer, pH 4.8

C. 1m M Pyridine/HCl - 6.0 M urea, pH 4.8

Wash Buffers

D. Phosphate buffered saline (PBS): 10 mM phosphate, 0.15 M NaCI, pH 7.2 (0.01% NaN_3 optional)

E. 1.4 M NaCl in PBS

F. 2.0 M GuaHCl in PBS

Elution Buffer

G. 3.5 M NaSCN - PBS

5.2 Preparation of Sample

Dissolve solid samples in PBS at 10-20 mg/ml. Dialyze or buffer exchange into coupling buffer A or B. (Buffer A is used for proteins with a pI $\geq$ 6; *e.g.* gamma globulins. Buffer B is used for proteins with a pI $\leq$ 6; e.g. albumin.) Use OD_{280} or the Bio-Rad Protein Assay to measure the level of protein soluble in the coupling buffer. If <50% of the original material is soluble, redialyze into coupling buffer C. If >50% is soluble, add any precipitated material as well as the soluble material to the reaction mixture. Liquid samples are dialyzed directly into the coupling buffers. It is advantageous to add the protein in as concentrated a form as possible and it may be necessary to concentrate dilute preparations.

5.3 Preparation of Gel

In a Buchner funnel, wash the required volume of gel with 3 bed volumes buffer E, 5 bed volumes water and 5 bed volumes of the coupling buffer which was used to prepare the sample.

5.4 Coupling

1. Transfer the required amount of gel with minimum additional volume of coupling buffer to an appropriate reaction vessel.
2. Add 5-20 mg protein for each ml of final packed bed volume. Larger amounts of protein (at 10-20 mg / ml) per ml gel bed result in higher capacity columns whereas lower amounts of protein added in as concentrated solutions as possible result in maximum coupling of the material.
3. Adjust pH of the slurry to the pH of the coupling buffer.
4. Stir gently for one half hour. Do not use a magnetic stirrer which may grind the gel.
5. Add 4-10 mg EDAC per ml gel volume. Use lower levels when smaller amounts of protein are used and higher levels when larger amounts of protein are used. The addition of more than 10 mg EDAC per ml gel will generally not increase coupling and may destroy the biological and antigenic activity of the product.
6. Maintain at the pH of the coupling buffer for one hour.
7. Rock or gently stir overnight.
8. Transfer the product to a column using a minimum amount of buffer E. Let settle.
9. If coupling buffer A or B was used, elute with 3 bed volumes of buffer E. Determine the approximate amount of protein coupled by measuring the total protein eluted with buffer E and subtracting this amount from the total added. (Bio-Rad Protein Assay, Catalog No. 500-0001)
10. If coupling buffer C was used, wash with a solution of 6 M urea, 0.15 M NaCl, 50 mM phosphate, pH 7.2, and dialyze an aliquot into PBS for protein determination. Dialysis is not necessary if the Bio-Rad Protein Assay is used.
11. Wash with 3 bed volumes of buffer F and 5 bed volumes PBS.

5.5 Applications

Table 3. DEAE Bio Gel A Gel

Application	Application Buffer	Elution Conditions	Reference
Purification of phosphocarrier protein of *Mycoplasma capricolum*	10 mM potassium phosphate, pH 7.4, 0.1 mM dithiothreitol, 0.1 mM EDTA	0-0.2 M KCl	4
Separation of lambda and kappa light chain proteins in urine	2 mM Tris-HCl, pH 8	0-15 mM NaCl	5
Displacement chromatography or serum preference	See reference		6
Purification of adenylate cyclase and removal of detergent	50 mM glycyl-glycine, pH 7.5, 3 mM dithio-threitol	Discontinuous NaCl gradient	7
Batch separation of hemoglobin A_1 and F from A_2	8.75 mM Tris-HCl pH 7.68, 6.36 mM Cl^-		8
Purification of gentamicin adenylyl transferase	10 mM Tris-HCl, pH 7.8, 0.125 mM EDTA, 0.3 mMDTT,1 mM Mg^{2+}	0-0.2 M $(NH_4)_2SO_4$	9

Application	Application Buffer	Elution Conditions	Reference
Purification of tubulin	50 mM PIPES buffer, 0.2 mM GTP, 50 mM NaCl	0.05-0.4 M NaCl	10
Purification of p-azido-benzoyl-CoA	20 mM HEPES, pH 7.4	0-150 mM NaCl	12
Purification of alkaline phosphatase from human milk	10 mM Tris-HCl, pH 7.4	30 mM NaCl	13
Purification of phospho-diesterases of rat renal cortex	25 mM potassium phosphate, pH 7.0, 0.1 mM EGTA	0.025-0.2 M potassium phosphate, pH 7.0	14
Purification of DNA polymerase	50 mM Tris-HCl, pH 7.5, 25 mM KCl, 1 mM DTT, 1 mM Na_3 EDTA, 10% glycerol, 1 mM amino-acetonitrile	Discontinuous KCl gradient	15
Purification of choline acetyl-transferase from *Drosophila melano-gaster*	10 mM citrate phosphate, pH 5.9	0-0.15 M NaCl	16

Application	Application Buffer	Elution Conditions	Reference
Purification of an exoglucanase from *Streptomyces flavogrisens*	10 mM phosphate, pH 7	0-0.2 M NaCl	17
Purification of DNA -polymerase activity from HeLa S3 cells	TDE buffer/10% glycerol	Stepwise 25,100, 300 mM KCl	15

Table 4. CM Bio-Gel A Gel

Application	Comments	Reference
Purification of phospho carrierprotein of *Myco-plasma capricolum*	10 mM NaAc buffer, pH, 0.1 mM DTT, 0.1 mM EDTA. Eluted with 0-0.3 M NaCl	4
Purification of carbonic anhydrase	Sulfonamide inhibitor p-amino-methylbenzene -sulfonamide coupled with CM Bio-Gel A gel using EDAC, pH 4.8	18

Application	Comments	Reference
Purification of somato-statin-like polypeptide	Applied in 1 M urea, 50 mM HEPES, pH 7.4. Eluted with linear gradient of increasing pH and ionic strength	19
Assay for dipeptidyl aminopeptidase III in RBC's	10 mM KPO_4, pH 6.5	20
Cystic Fibrosis muco-ciliary inhibitor	10 mM ammonium acetate, pH 5.0, eluted with 10 mM to 50 mM ammonium acetate, 0.2 M and 1.0 M ammonium acetate	21

5.6 Storage

When not in use, store the DEAE and CM Bio-Gel A ion exchange supports in 0.05% NaN_3 as a bacteriostat.

5.7 Shelf Life

The Bio-Gel A ion exchange supports are stable for at least 1 year when stored sealed in the original container at 4 °C.

5.8 Technical Assistance

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

References

1. Harris, E. L. V. and Angal, S., Protein Purification Methods, a Practical Approach, IRL Press, Oxford, (1989).
2. Scopes, R. K., Protein Purification, Principles and Practice, (Second Edition), Springer-Verlag, New York, (1987).
3. Snyder, L. R. and Kirkland, J. J., Introduction to Modern Liquid Chromatography, (Second Edition), John Wiley & Sons, Inc., New York, (1979).
4. Ullah, A. H. J. and Cirillo, V. P., *Journal of Bacteriology*, **127**, 1298 (1976).
5. Dalal, F. R. and Winsten, S., *CUn. Chem.*, **25**,1(1979).
6. Peterson, E. A., *Anal. Biochem.*, **90**, 767 (1978).
7. Johnson, R. A., Garbers, D. L. and Pilkis, S. J., *J. Supramolecular Structure*, **4**, 205 (1976).
8. Morin, L. G., *Clin. Chem.*, **21**, 1490 (1975).
9. Goldman, R. R. and Northrop, D. B., *Biochem. Biophys. Res. Commun.*, **66**, 1408 (1975).
10. Garland, D. L., *Biochemistry*, **17**, 4266 (1978).
11. Khalifah, R. G., Strater, D. J., Bryant, S. H. and Gibson, S. M., *Biochemistry*, **16**, 2241 (I 977)
12. Myers, D. E. and Utter, M. E;, *Anal. Biochem.*, **112**, 23 (1981).
13. Hamilton, T. A., Gornicki, S. Z. and Sussman, H. H., *Biochem. J.*, **177**, 197 (1979).
14. Strewler, G. J., Manganiello, V. C. and Vaughn, M., *J. Biol. Chem.*, **253**, 390 (1978).

15. Lamothe, P., Baril, B., Chi, A., Lee, L. and Baril, E., *Proc. Natl. Acad. Sci. USA*, **78**, 4723 (1981)
16. Slemmon, J. R., Salvaterra, P. M., Crawford, G. D. and Roberts, E., *J. Biol. Chem.*, **257**, 3847 (1982).
17. Macken~ie, C. R., Bilous, D. and Johnson, K. G., *Can. J. Microbiol.*, **30**, 1171(1984).
18. Khalifah, R. G., Strader, D. J., Bryant, S. H. and Gibson, *S. M., Biochemistry*, **16**, 2241 (I 977) .
19. Spiess, J., Villarreal, J. and Vale, W., *Biochemistry*, **20**, 1982 (1981).
20. Jones, T. H. D. and Kapralou, A., *Anal. Biochem.*, **119**, 418 (1982).
21. Carson, S. D. and Bowman, B. H., *Pediatric Res.*, **16**, 13 (1982).

Ordering Information

Catalog Number	Product Description	Type
153-0740	**DEAE Bio-Gel A Gel, 250 ml**	Agarose based weak anion exchanger. Fractionation of high molecular weight ionic substances such as immunobglobulins, enzymes and other proteins and nucleic acids.
153-0840	**CM Bio-Gel A Gel, 250 ml**	Agarose based weak cation exchanger. Fractionation of high molecular weight ionic substances such as immunobglobulins, enzymes and other proteins and nucleic acids.
Desalting and sample preparation:		
150-0738	**Bio-Gel P-6DG Desalting Gel, 100 g**	Rapid protein and peptide desalting.
150-0739	**Bio-Gel P-6DG Desalting Gel, 1 kg**	

Catalog Number	Product Description	Type
732-2010	**Econo-Pac 10DG Desalting Columns, 30 columns of 10 ml**	Prepacked Bio-Gel P-6DG. For desalting up to 3.3 ml samples
732-0011	**Econo-Pac P6 Cartridge, 5 ml**	For desalting of up to 2 ml samples
732-6002	**Bio-Spin 6 Chromatography Columns, 25**	For desalting of samples up to 100 µl