CM Sepharose Pre-Packed Column

Catalog # 9080

For Research Use Only - Not Human or Therapeutic Use

DESCRIPTION:	CM: 6% cross-linked Sepharose
APPLICATION:	Separation and purification of all charged biomolecules (e.g. proteins, peptides, nucleic acids, etc.)
QUANTITY:	5 ml (resin volume)
FORM:	Suspension in 20% ethanol (50% slurry)
ION CAPACITY:	Weak cation exchange, 90-130 µmol H^+/ml media
pH RANGE:	pH 6 - 10
STORAGE:	4 - 30 degrees C in 20% ethanol (DO NOT FREEZE)
STABILITY:	1 year
COLUMN DIMENSIONS:	20 cm x 2 cm
NOTES:	N/A
REFERENCES:	N/A

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INTRODUCTION

Each protein has a net charge called the isoelectric point (pI) as determined by its primary amino acid sequence. This charge property allows proteins to selectively bind to resins with opposite charges in ion-exchange chromatography. CM is a weak cation exchange media containing a carboxymethyl functional group [-O-CH_2COO^-]. Chondrex, Inc. offers prepacked CM Sepharose columns (5 ml) which are suitable for separating and purifying positively charged biomolecules including proteins, peptides, and nucleic acids. Recommended buffers to use in cation exchange chromatography are listed below in Tables 1 and 2.

Table 1. Anion Exchange Buffer*

pH range	Buffer salt	Concentration (mM)	Counterion	pKa (25 Degrees C)
4.3 - 5.3	N-Methylpiperazine	20	Cl^-	4.75
4.8 - 5.8	Piperazine	20	Cl^- or $HCOO^-$	5.33
5.5 - 6.5	L-Histidine	20	Cl^-	6.04
6.0 - 7.0	bis-Tris	20	Cl^-	6.48
6.2 - 7.2	bis-Tris propane	20	Cl^-	6.65
7.3 - 8.3	Triethanolamine	20	Cl^- or CH_3COO^-	7.76
7.6 - 8.6	Tris	20	Cl^-	8.07
8.0 - 9.0	N-Methyl-diethanolamine	20	SO_4^{2-}	8.52
8.0 - 9.0	N-Methyl-diethanolamine	50	Cl^- or CH_3COO^-	8.52
8.4 - 9.4	Diethanolamine	50	Cl^-	8.88
8.4 - 9.4	Propane 1,3-Diamino	20	Cl^-	8.88
8.6 - 9.6	bis-Tris propane	20	Cl^-	9.10
9.0 - 10.0	Ethanolamine	20	Cl^-	9.50
9.2 - 10.2	Piperazine	20	Cl^-	9.73
10.0 - 11.0	Propane 1,3-Diamino	20	Cl^-	10.55
10.6 - 11.6	Piperidine	20	Cl^-	11.12

Table 2. Cation Exchange Buffer*

pH range	Buffer salt	Concentration (mM)	Counterion	pKa (25 Degrees C)
1.4 - 2.4	Maleic acid	20	Na^+	1.92
2.6 - 3.6	Methyl malonic acid	20	Na^+ or Li^+	3.07
2.6 - 3.6	Citric acid	20	Na^+	3.13
3.3 - 4.3	Lactic acid	50	Na^+	3.86
3.3 - 4.3	Formic acid	50	Na^+ or Li^+	3.75
3.7 - 4.7	Succinic acid	50	Na^+	4.21
4.3 - 5.3	Acetic acid	50	Na^+ or Li^+	4.75
5.1 - 6.1	Succinic acid	50	Na^+	5.64
5.2 - 6.2	Methyl malonic acid	50	Na^+ or Li^+	5.76
5.6 - 6.6	MES	50	Na^+ or Li^+	6.27
6.7 - 7.7	Phosphate	50	Na^+	7.20
7.0 - 8.0	HEPES	50	Na^+ or Li^+	7.56
7.8 - 8.8	BICINE	50	Na^+	8.33

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NOTE 1: Improper buffer (type and concentration) may interfere with the purification separation effects (e.g., resolution, capacity, pH fluctuations, etc.).

NOTE 2: The pH of the buffer used should be within the ideal pH range of the ion exchange media.

SAMPLE PROTOCOL (Lysozyme Purification)

Buffer Preparation:

- Equilibration Buffer: 50 mM phosphate buffer, pH 6.8
- Elution Buffer: 50 mM phosphate buffer, 1M NaCl, pH 6.8

  NOTE: Depending on the proteins, the buffer system and pH can vary.

Sample Preparation:

- The buffer of the samples should be the same as the equilibration buffer.

  NOTE: the buffer can be exchanged by dialysis or diafiltration.
- Clarification of the samples by centrifugation or filtration.

Purification Protocol:

1. Ensure the resin in the column is packed at the bottom of the column.
2. Remove the cap, attach a 3-way stopcock, open the valve, and drain the storage solution in the column.
3. Keep the stopcock valve open and equilibrate the column with 50 ml of equilibration buffer.
4. When equilibrating is complete, drain the column until no buffer remains in the resin and close the stopcock valve.
5. Open the stopcock valve and load the sample onto the column.
6. Pool the flow through sample.
7. Drain the column until no sample remains in the resin and close the stopcock valve.
8. Open the stopcock valve and wash the column with equilibration buffer.
9. Collect 5 to 10 tubes of 5 ml fractions. Keep collecting until the A280 OD value stabilizes.
10. Drain the column until no buffer remains in the resin and close the stopcock valve.
11. Open the stopcock valve and elute the column with elution buffer.
12. Collect 5 to 10 tubes of 5 ml elution fractions.
13. Check the A280 OD values of each fraction.

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14. Drain the column until no buffer remains in the resin and close the stopcock valve.
15. Combine all positive fractions into a single purified sample.

    NOTE: if needed, concentrate the sample by ultrafiltration and then dialyze to change the buffer.
16. Open the stopcock valve and wash the column with 25 ml of distilled water.
17. Drain the column until no water remains in the resin and close the stopcock valve.
18. Open the stopcock valve and wash with 25 ml of distilled water containing 20% ethanol until the liquid level is several millimeters above the top of the resin bed (do not allow the resin to dry out).
19. Close the stopcock, replace the cap, and ensure all connections are tight and sealed.
20. Store at 4 - 30 degrees C.

Regeneration Protocol

When the elution speed gets slow due to protein/lipid contamination, it is necessary to regenerate the CM Sepharose resin.

1. Wash the column with 25 ml of distilled water.
2. Wash the column with 50 ml of 0.1 M NaOH to remove contaminant proteins/lipids.
3. Wash the column with 50 ml of 0.1N HCl
4. Wash the column with 50 ml of 0.1M Tris-HCl, pH 7.4
5. Wash the column with 25 ml of distilled water.
6. Wash the column with 25 ml of distilled water containing 20% ethanol. Stop when the liquid level is still several millimeters above the top of the resin bed (do not allow the resin to dry out).
7. Close the stopcock, replace the cap, and ensure all connections are tight and sealed.
8. Store at 4 - 30 degrees C.

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