



Life Sciences

Application Note

USD 2410

Purification of Mouse IgM
from Cell Culture Supernatant
by Cation Exchange Chromatography
on CM Ceramic HyperD[®] F Sorbent

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What this Study Demonstrates

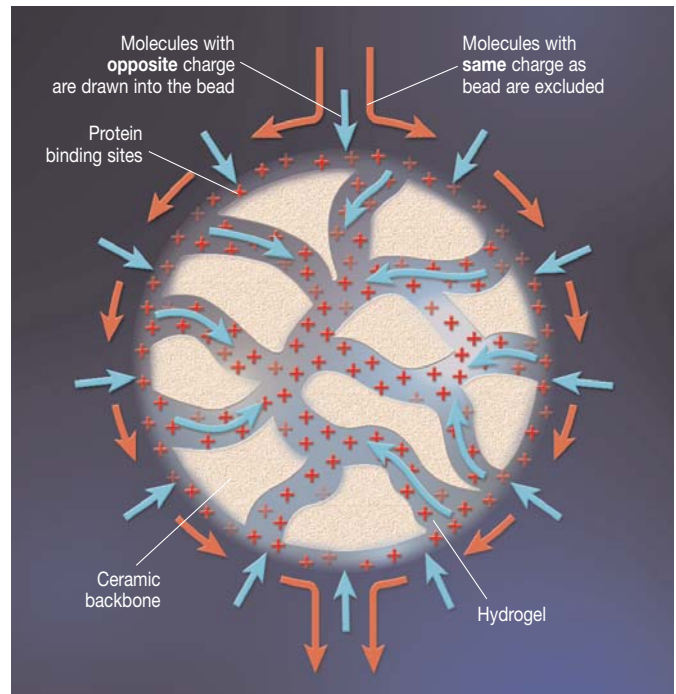
This study on Cation Exchange Chromatography demonstrates that CM Ceramic **HyperD** F sorbent is an effective first capture step for mouse IgM from albumin-rich cell culture supernatants. The specific IgM single-step capture described in this study resulted in a 77% pure IgM with a 68% yield.

The data shows that binding pH, working flow rate, and step-elution are important parameters that must be optimized on a case-to-case basis. To increase purity, additional chromatographic steps (such as gel filtration) can be easily implemented after the cation exchange step. Finally, CM Ceramic **HyperD** F is a fully scalable sorbent that can be operated at high flow rates in multi-liter columns for preparative separations, using conventional low pressure chromatography equipment.

1. Introduction

IgM are very large molecules difficult to purify because of their size and risk of aggregation. CM Ceramic **HyperD** F sorbent was initially developed for the capture and purification of antibodies (typically IgG) from complex feedstocks. This study demonstrates that CM Ceramic **HyperD** F can also be used for the capture of IgM from concentrated, albumin-containing cell culture supernatants. Optimization principles and results are presented.

"Gel-in-a-shell" design of **HyperD** sorbent.



Ceramic **HyperD** sorbents deliver outstanding dynamic capacity and exceptional dimensional stability. This translates into unsurpassed productivity.

2. Objective

Design a scalable single-step capture of mouse IgM from cell culture supernatant by means of cation exchange chromatography on CM Ceramic **HyperD** F sorbent.

3. Materials and Methods

- **Samples:** 100-fold concentrated mouse cell culture supernatant (CCS), containing albumin and transferrin from the cell culture medium (total protein concentration of 100 mg/mL, including mouse IgM [3.2 mg/mL]).
- **Chromatography:** Cation exchange on CM Ceramic **HyperD F** sorbent (Pall), equilibration in 100 mM sodium acetate, pH 5.2, 5.5, or 5.7. Elution by NaCl gradient (see data). The concentrated CCS was loaded directly on the column (0.46 cm I.D. x 5 cm height) after a 4-fold dilution. Runs were performed on an ÄKTA* Explorer 100 (GE Healthcare*).
- **Analytics:** Fractions were analyzed by SDS-PAGE (12% polyacrylamide gels). IgM purity was assessed by SEC HPLC on a TSKgel* G4000SWXL column (Tosoh Bioscience). IgM yields were estimated using an HPLC assay developed on the above column with a standard bovine IgM solution (Sigma).

Table I. Main properties of CM Ceramic HyperD F sorbent

Average particle size	50 µm
Dynamic binding capacity for IgG, 10% breakthrough, 200 cm/h	≥ 60 (mg/mL) ⁽¹⁾
Amount of ionic groups	250 - 400 (µeq/mL)
Working pH	2-12
Cleaning pH	1-14
Volumes changes due to pH and ionic strength	Non compressible
Pressure resistance	70 bar (1,000 psi)

(1) Sample: 5 mg/mL hu IgG in 50 mM sodium acetate, 100 mM NaCl, pH 4.7.

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4. Chromatography Optimization Methodology on CM Ceramic HyperD F Sorbent

4.1. Screening of Binding Conditions

Binding of antibodies to cation exchangers is generally achieved between pH 4.5 and 6.0. In the case presented in this note, due to the high albumin concentration in the feedstock, the capture of IgM on CM Ceramic HyperD F sorbent was tested at three different binding pH's, chosen above the isoelectric point of albumin (approximately 5). The objective was to limit the binding of the albumin molecules to the negatively charged sorbent, while achieving an efficient binding of the IgM. The selected pH were therefore pH 5.2, 5.5 and 5.7. The elution was performed through a linear positive salt gradient.

- Binding at pH 5.2:** As shown in Figure 1, capture at pH 5.2 resulted in a low purity (24 %) of the IgM and contamination by albumin originating from the cell culture medium.
- Binding at pH 5.5:** As shown on the chromatogram in Figure 2, the IgM purity was improved to 61 % (SEC-HPLC estimation): the interaction of albumin with the sorbent decreased due to the increase of binding pH. The contaminating albumin was mainly desorbed at higher salt concentration than the IgM.

- Binding at pH 5.7:** This pH value was too high (too close to the IgM isoelectric point) to allow an efficient binding of the IgM.

The results are summarized in Table II. Finally, pH 5.5 was selected as the optimum binding pH, allowing to achieve the highest purity in the elution pool. However, the IgM yield was still low (29 %), requiring further optimization of the elution mode.

Table II. Summary of the purification factors and yields obtained during binding conditions screening for the purification of a concentrated mouse IgM feedstock on CM Ceramic HyperD F sorbent.

Step	[IgM] (mg/mL)	Purity (%)	Purification factor	Yield (%)
Load	0.8	5	-	100
Binding pH 5.2 Elution pool	0.8	24	5	80
Binding pH 5.5 Elution pool	0.4	61	12	29
Binding pH 5.7 Elution pool	0.1	55	11	8

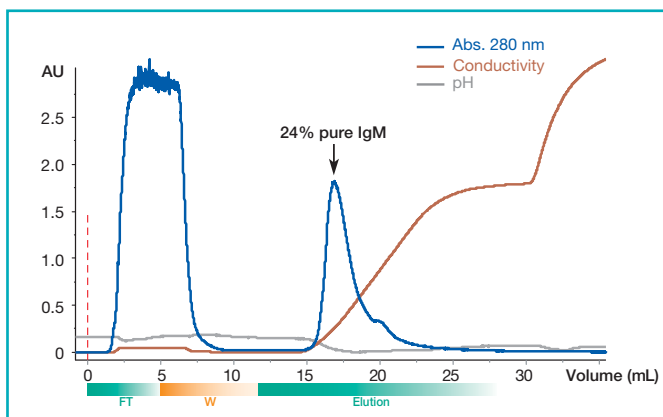


Figure 1. Purification of a concentrated mouse IgM feedstock on CM Ceramic HyperD F sorbent with a binding at pH 5.2.

Load: 5mL after a 4-fold dilution; Equilibration + Wash: **Buffer A:** 100 mM sodium acetate, pH 5.2; Elution: 0 – 50 % B (10 CV) followed by 100 % B (6 CV); **Buffer B:** 100 mM sodium acetate, pH 5.2 + 1.5 M NaCl; Flow rate: 150 cm/h (residence time: 2 min.).

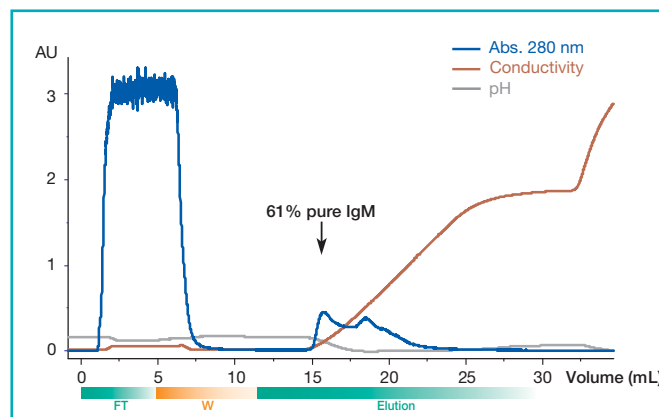


Figure 2. Purification of a concentrated mouse IgM feedstock on CM Ceramic HyperD F sorbent with a binding at pH 5.5.

Load: 5 mL after a 4-fold dilution; Equilibration + Wash: **Buffer A:** 100 mM sodium acetate pH 5.5; Elution: 0 – 50 % B (12 CV) followed by 100 % B (6 CV); **Buffer B:** 100 mM sodium acetate, pH 5.5 + 1.5 M NaCl; Flow rate: 150 cm/h (residence time: 2 min.).

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4.2. Improving IgM Yield and Purity by Optimization of Elution Conditions

After binding at pH 5.5, a three-step elution sequence was implemented with respectively 0.1 M, 0.2 M, and 0.3 M NaCl solutions, as described in Figure 3. The load, as well as the linear flow rate were both reduced.

Results in Figure 4 and Figure 5 confirmed that at pH 5.5, most of the contaminating albumin was unretained and was found in the column flowthrough and wash fractions. The IgM was eluted in the first fraction (elution pool E1, using 0.1 M NaCl) with an increased purity and yield (purity 77 %, yield 68 %). The other fractions (E2 and E3, 0.2 and 0.3 M NaCl) contained the residual contaminating albumin.

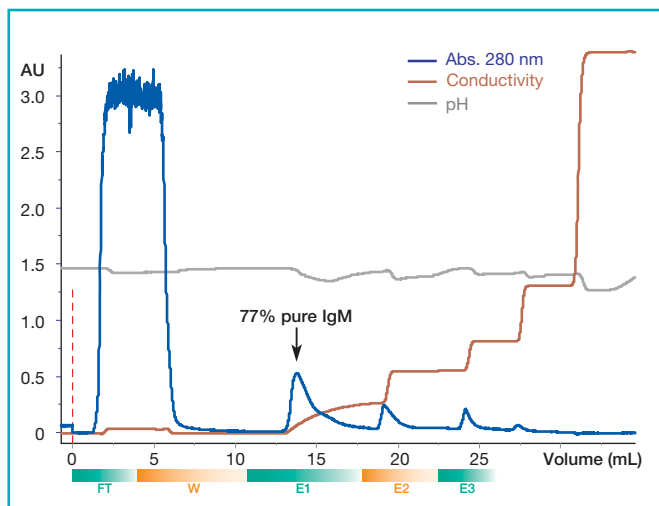


Figure 3. Purification of a concentrated mouse IgM cell culture supernatant on CM Ceramic **HyperD** F sorbent. Loading at pH 5.5 and step-gradient elution with NaCl.

Load: 4 mL after a 4-fold dilution
 Equilibration + Wash: 100 mM sodium acetate, pH 5.5
 Elution: E1 Equilibration buffer + 0.1 M NaCl
 E2 Equilibration buffer + 0.2 M NaCl
 E3 Equilibration buffer + 0.3 M NaCl
 Flow rate: 43 cm/h (residence time: 7 min.)

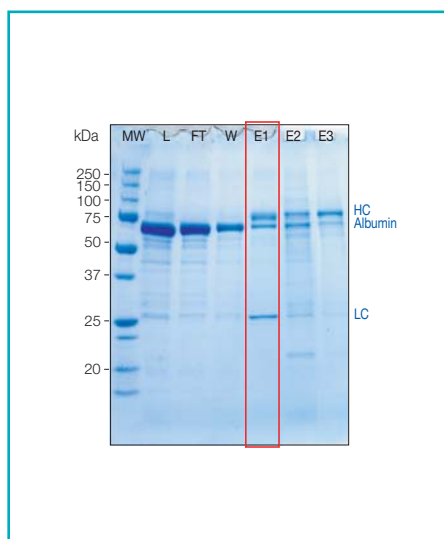


Figure 4. SDS-PAGE analysis in reduced conditions.

L = Load; FT = Flowthrough; W = Wash;
 E1...3 = Elution pool E1 ... E3.

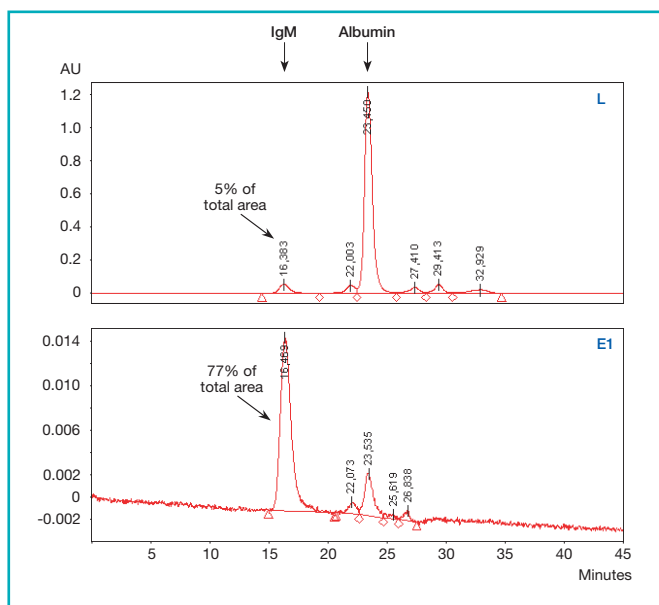


Figure 5. Analysis of the crude concentrated IgM feedstock (L) and of the elution pool (E1) on a TSKgel® G4000SWXL column.

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References

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Ordering Information

Sorbent	Pack size	Part Number
CM Ceramic HyperD F	5 mL	20050-084
	25 mL	20050-035
	100 mL	20050-027
	1 L	20050-019
	5 L	20050-050
	10 L	20050-043



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