Acidic IgM Purification with the Strong Anion Exchanger, Nuvia HP-Q Resin

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Abstract

IgM antibodies are one of the fastest growing groups of diagnostic and therapeutic candidates. Unlike IgGs, IgMs cannot be purified by affinity chromatography due to their physiochemical properties. Furthermore, the large size of IgMs presents an additional purification challenge. Bio-Rad has developed a new strong anion exchange (AEX) resin — Nuvia HP-Q — to overcome the multiple issues faced when purifying large biomolecules. We show the purification of an acidic IgM antibody with capture purification using Nuvia HP-Q and polish purification using mixed-mode CHT Ceramic Hydroxyapatite Media. The capture step removed process-related impurities, while polishing with ceramic hydroxyapatite depleted these impurities further. The studies resulted in >99% IgM product purity, as measured by size exclusion chromatography (SEC) and SDS-PAGE analysis. This purity makes the IgM suitable for diagnostic applications. Additionally, this purification strategy is scalable and has potential for pharmaceutical applications.

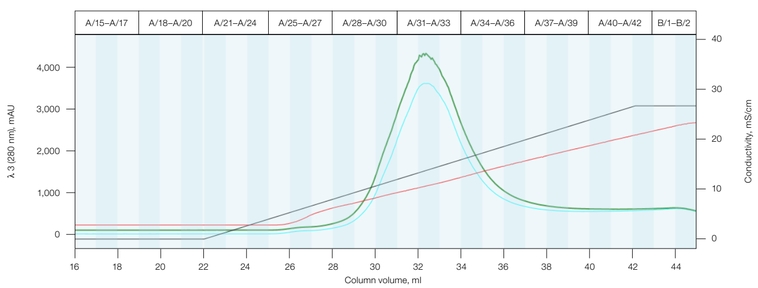
Introduction

IgM molecules are penta- and hexameric protein multimers with a size of 900–1,000 kD. Recombinant IgM antibodies are used in diagnostic and some therapeutic applications. IgM purification presents multiple challenges. Affinity purification with Protein A resins is not an option. The large size of IgMs has direct impact on several process parameters in chromatographic purification. IgM's dynamic binding capacity (DBC) is significantly reduced compared to smaller proteins, due to their inability to penetrate small pores and insufficient utilization of the pore volume. The slow diffusion of these large molecules also affects all mass-transport-dependent chromatography steps.

To overcome these large molecule purification issues, Bio-Rad developed the Nuvia HP-Q AEX Resin. It is built on the hydrophilic UNOsphere epoxide base bead that provides fast mass transfer kinetics and low nonspecific binding. Its pore size is optimized for easy accessibility and adsorption of large biomolecules such as IgMs, VLPs, viruses, and PEGylated proteins. The internal spacer length and ligand density facilitate optimal binding of the large biomolecules even at high flow rates. Here, we present the results obtained by purifying an acidic IgM antibody with a pI of 6.7 on Nuvia HP-Q and CHT-II-40 Media.

Capture Purification of an Acidic IgM Antibody with AEX Resin, Nuvia HP-Q

The acidic IgM was expressed in a HEK transient expression system, and had a calculated pI of 6.7. Approximately 400 ml of the cell culture supernatant with ~2 mg of IgM antibody was adjusted to pH 7.5 and loaded onto a Nuvia HP-Q Foresight Column. Elution of the bound IgM was carried out with a linear gradient to 200 mM NaCl (Figure 1). The IgM peak is seen with the 280 nm probe between A28 and A37. These fractions were pooled together to run on the CHT-II-40 column for subsequent polishing of the target protein.

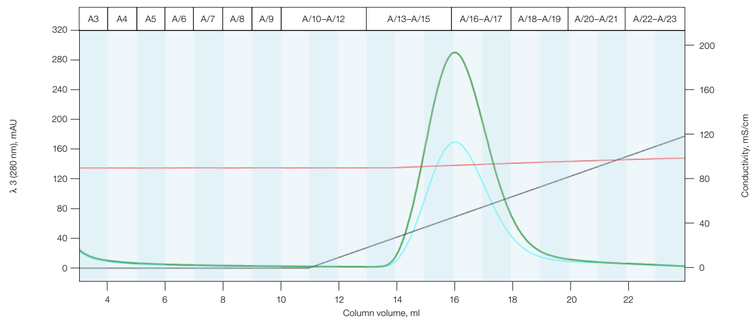


Column:	Nuvia HP-Q, 1 ml Foresight
Equilibration:	10 CV of 20 mM Na_2HPO_4/NaH_2PO_4, pH 7.5 (buffer A)
Sample:	400 ml cell culture supernatant adjusted to pH 7.5 with 800 ml equilibration buffer
Flow rate:	120 cm/hr
Wash:	20 CV buffer A
Elution:	20 CV linear gradient from 0–20% buffer B
Buffer A:	20 mM Na_2HPO_4/NaH_2PO_4, pH 7.5
Buffer B:	1 M NaCl, 20 mM Na_2HPO_4/NaH_2PO_4, pH 7.5

Fig. 1. Capture purification of IgM on Nuvia HP-Q. Chromatography run of IgM on Nuvia HP-Q with 0–200 mM NaCl linear gradient. λ 2 (260 nm) (■); λ 2 (260 nm) (■); conductivity (■), %B (■).

Polish Purification of the Acidic IgM Antibody with Mixed-Mode Media, CHT-II-40

CHT Media, Type II, 40 µm (CHT-II-40) was used for polishing purification. The pH of the pooled fractions from A28 to A37 from the capture step was adjusted to 7.0 with 0.1 M H_3PO_4. The column was pre-equilibrated and a linear phosphate gradient to 400 mM NaPi was applied to elute the purified IgM (Figure 2).



Column:	CHT-II-40, 1 ml Foresight
Equilibration:	10 CV of 1 M NaCl, 20 mM Na_2HPO_4/NaH_2PO_4, pH 7.5
Sample load:	Nuvia HP-Q elution pool, supplemented with NaCl, pH adjusted to 7.0 using diluted H_3PO_4
Wash:	10 CV buffer C
Elution:	20 CV linear gradient from 0–100% buffer D
Buffer C:	1 M NaCl, 20 mM Na_2HPO_4/NaH_2PO_4, pH 7.0
Buffer D:	1 M NaCl, 400 mM Na_2HPO_4/NaH_2PO_4, pH 7.0

Fig. 2. Polish purification of IgM on CHT-II-40. Chromatography run of the previously eluted IgM on CHT-II-40 with a 20–400 mM phosphate and 1 M NaCl gradient. λ 2 (260 nm) (■); λ 2 (260 nm) (■); conductivity (■), %B (■).

Results

The resulting IgM after the polish purification was run on a non-reducing (Figure 3A) and a reducing gel (Figure 3B) for purity analysis. The CHT-II-40 eluate showed a single band of IgM in the non-reducing gel and two bands for the heavy and the light chains on the reducing gel. Contaminant bands were not seen in these lanes.

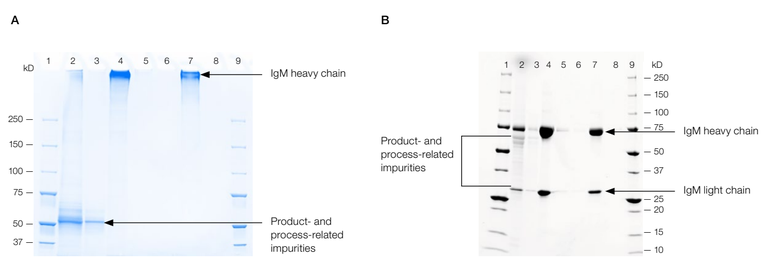


Fig. 3. Reduced SDS-PAGE analysis of IgM purified from the Nuvia HP-Q and CHT-II-40 Columns.

The purity was further confirmed by SEC analysis. Contaminant peaks were seen on the SEC plot with the Nuvia HP-Q eluate (Figure 4A), but only a single sharp peak for IgM was seen on the SEC plot with the CHT-II-40 eluate (Figure 4B).

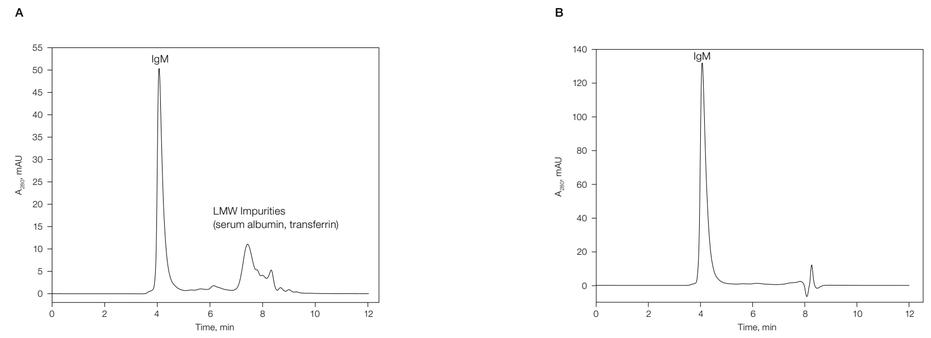


Fig. 4. SEC analysis of the Nuvia HP-Q eluate (A) and CHT-II-40 eluate (B). Analysis was performed on a Dionex UltiMate 3000 System using a MAbPac SEC-1 Column. A sample volume of 3 µl (Nuvia HP-Q eluate) or 10 µl (CHT-II-40 eluate) was injected. Analytical separation was achieved in isocratic mode using 50 mM Na_3PO_4, 200 mM arginine, 300 mM NaCl, pH 6.8 as running buffer at 0.8 ml/min for 12 minutes.

Analysis of the contaminant levels in each of the samples showed a reduction in the DNA and host cell protein (HCP) content after the Nuvia HP-Q purification. Further purification with CHT-II-40 resulted in further minimizing these contaminants as shown in Table 1.

Table 1. Final purification data.

	Purity, %*	Recovery, %*	DNA, pg/µl**	HCP, ppm***
Culture supernatant	20	—	2,332	>200,000
Nuvia HP-Q EP	63	83	1,187	21,455
CHT-II-40 EP	>99	77	Not detectable	109

EP, elution pool
* Determined by SEC-HPLC
** DNA content determined by Quant-iT PicoGreen Assay
*** HCP content determined by Cygnus HEK293 Kit

Conclusions

This non–affinity based platform approach using the strong AEX Nuvia HP-Q Resin gives a >99% pure IgM. This meets the purification requirements for diagnostic IgMs manufacturing and shows high potential for pharmaceutical purification. The process involves only two steps and requires a narrow range of optimized conditions, making it suitable for scalable purification. Using this state-of-the-art media with high capacity and selectivity for large biomolecules also helps overcome the affinity-based challenges of low binding and/or recovery during IgM purification.

Acknowledgements

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References

Gagnon et al. (2008). Purification of IgM monoclonal antibodies. biopharminternational.com/purification-IgM-monoclonal-antibodies, accessed June 3, 2018.

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