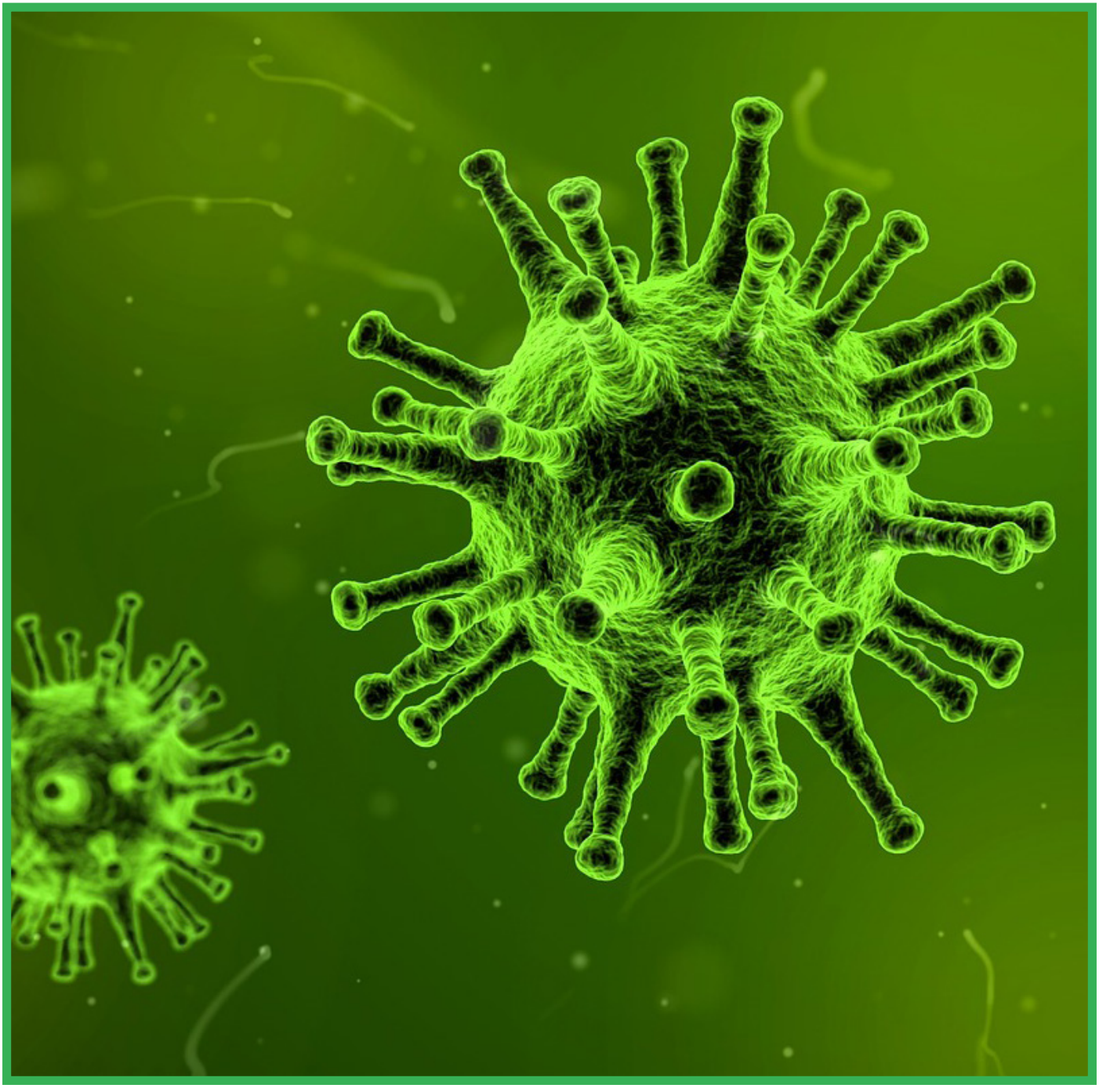
Virus Purification Strategies

Improved virus purification strategies with advanced chromatographic techniques and optimal resin selection



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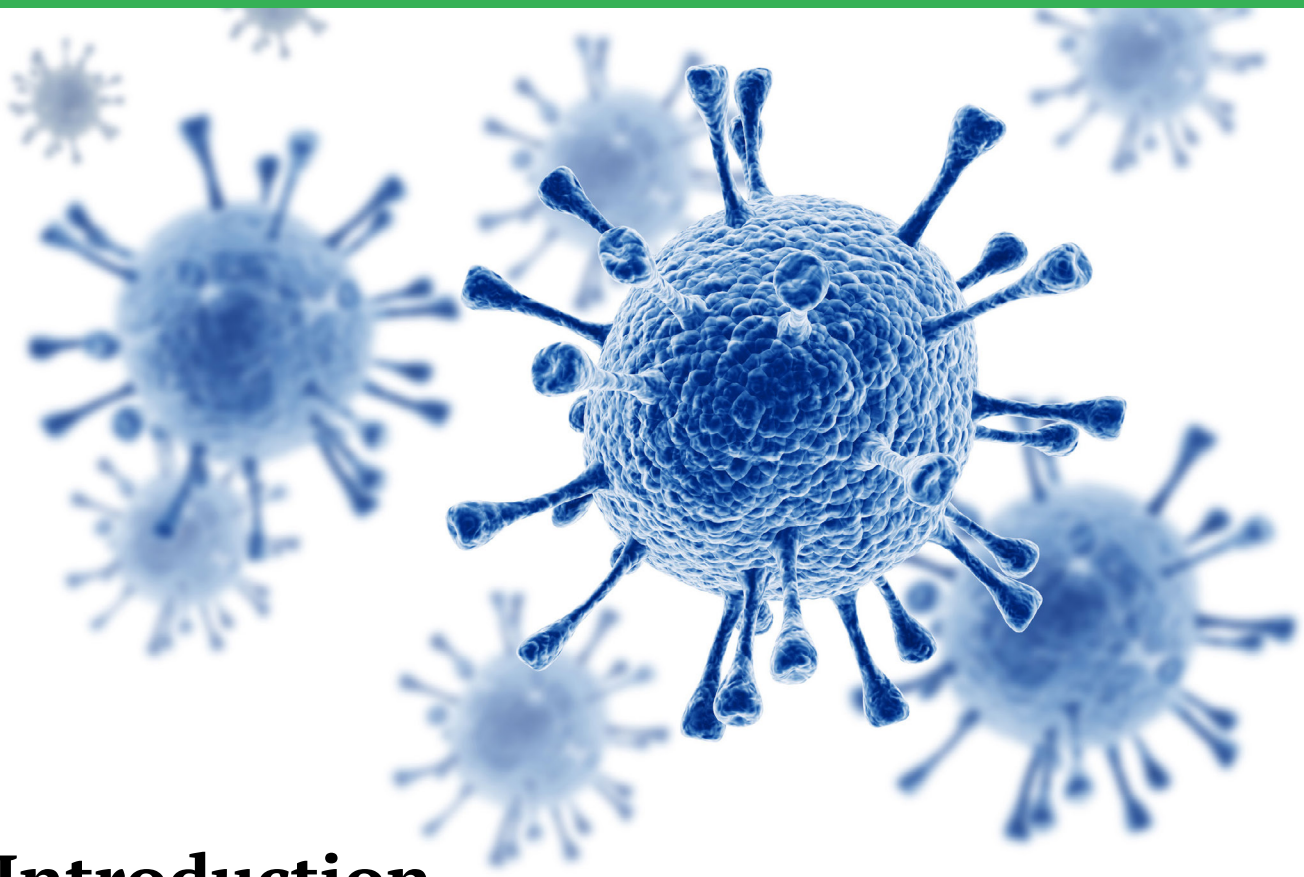

Introduction

Viral particles form an ever-increasingly important class of biologics, with applications in vaccine development, disease prevention and as delivery mechanisms for gene therapy and the treatment of diseases such as cancer.

In addition to their important biotherapeutic potential, biological study and characterization of virus infectivity, reproduction and host immune system evasion are of continued importance. Ensuring that virus production and purification are of the highest quality for effective recovery of particles is therefore vital for both research and therapy.

In this application eBook, we look at methodologies for optimized virus process purification to achieve increased productivity and improved economics with chromatography systems and innovative resins.

Contents

Challenges in virus production

Viruses can be large and complex, both in terms of genetic material and surface diversity, which introduces barriers to their production and purification. Traditional methods of purification, such as ultracentrifugation, precipitation and filtration are inefficient, producing virus recovery of variable quality, quantity and infectivity. This, therefore, falls short of the consistent purity standards required for biotherapeutics.

In order to overcome these challenges, column chromatography has become an increasingly useful technique to improve

recovery and impurity removal while increasing process productivity and maintaining virus infectivity. Designed to meet the optimization requirements for different virus particle sizes, Bio-Rad's chromatography systems and wide range of resins enable process-scale virus purification.

Choosing the right chromatography media

High-capacity or high-affinity chromatographic resins bind viruses through one or more mechanisms and can be fine-tuned to modify binding capacity and selectivity through resin architecture and surface design. Bio-Rad offers a variety of resins for this purpose:

- High-performance strong anion exchange resins, such as Nuvia HP-Q, are well suited for downstream purification of large biomolecules at fast flow rates, without loss in dynamic binding capacity and recovery.

- CHT Ceramic Hydroxyapatite Type II Media consists of calcium affinity and cation exchange interactions, providing high purity, recovery, and viral activity of varying size viruses. This has been shown in a mammalian case study.

- In this application note, we see how Ceramic Hydroxyapatite XT Media is able to recover over 75% of the target virus while reducing over 90% of contaminating proteins, demonstrating robust performance with healthy process economics, even in a single-step purification protocol.

Method development

Although significant periods of time may be needed in method development, this enables improved chromatography performance and productivity. From initial capture to polishing steps, it is important to increase total recovery and purity while also using conditions that make downstream processes easier.

While purification resin selection is an important step in method development, for simple, fast and reproducible virus purification, remember to consider resin lifespan, stability over repeated uses, scalability and single-step purification functionalities for improved process economics. To optimize your process development, consider prepacked columns, RoboColumn Units, plates or bulk resin to screen resin types and evaluate your design space.

Single-Step Influenza and Dengue Virus Purification with Mixed-Mode Media

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Introduction

Virus preparations have been used as vaccines for over two hundred years, and more recently for gene therapy platforms. For gene delivery, conventional viral purification methods such as density gradient ultracentrifugation and precipitation can be difficult to scale and may not meet the purity standards required for a therapeutic. Consequently, chromatography has emerged as an advanced tool to meet large-scale therapeutic virus purification requirements. Viruses bind to chromatographic resins by electrostatic interactions.

We have previously demonstrated mammalian virus purification with the mixed-mode CHT Ceramic Hydroxyapatite Media (Kurosawa et al. 2014). CHT Media are a group of mixed-mode calcium affinity/cation exchange supports. The high negative surface charge on many viruses allows them to bind tightly to CHT calcium sites, permitting significant purification with high (>80%) yields. Such tight binding has also been utilized to provide robust viral clearance, which can be used to ensure viral safety during biomanufacturing (ICH Expert Working Group 1999, Moritz 2005).

CHT XT is the newest addition to the CHT family of media. It has been designed for superior physical robustness to ensure it can be used repeatedly over a large number of cycles. It maintains the unique separation properties of CHT for aggregate removal and monoclonal antibody purification. In order to test its virus purification capabilities, we performed single-step purifications of influenza and dengue viruses using CHT XT Media.

Materials and Methods

The viruses used in this study are shown in Table 1.

Table 1. Virus type and size.

Virus	Genome	Envelope	Size, nm
Influenza	ssRNA	+	80–120
Dengue	ssRNA	+	50

Virus Production

Influenza virus: Strain A/California/07/2009 was cultured on MDCK cells in minimal essential medium (MEM; Gibco) containing 10% fetal bovine serum (FBS) and L-glutamine in 225 cm^2 flasks. When the cells were almost confluent, influenza virus was inoculated onto the cell monolayer at a 1:3,000 dilution in 75 ml of D-MEM/F-12 with trypsin, penicillin, and streptomycin (flu culture medium). The supernatant was harvested on days 3, 4, and 11 and clarified through 0.45 µm filters.

Dengue virus: Type 2 strain ThNH7/93 was cultured on C6/36 cells. Cells were grown in 225 cm^2 flasks that were precoated with poly-L-lysine at 100 µg/ml in phosphate buffered saline (PBS). Cells were cultured in Minimum Essential Medium Eagle (modified) (modified EMEM, MP Biomedicals) containing 10% FBS at 28°C for 1 week. After the cells reached confluence, dengue virus type 2 was inoculated at a 1:1,000 dilution onto the cell monolayer in 75 ml of modified EMEM containing 0.5% FBS and MEM Vitamin Solution (Invitrogen Corporation) and cultured at 28°C. The medium was changed at day 3 and culture fluid (about 75 ml) was collected at day 7. The culture fluid was filtered through a 0.45 µm filter to remove cells and large cell debris.

Chromatography

CHT XT Media was packed into 4.6 x 35 mm columns (0.58 ml bed volume) and viral purification was performed at a flow rate of 360 cm/hr as shown in Table 2 for influenza and Table 3 for dengue. All experiments were carried out in triplicate and representative data are shown.

Table 2. Influenza purification protocol.

Step	Mobile Phase	pH	Volume, ml
Pre-wash	1 M NaOH		5
Wash	600 mM sodium phosphate	7.2	10
Equilibration	10 mM sodium phosphate	7.2	10
Wash	24% of 600 mM sodium phosphate	7.2	10
Sample loading and washing	24% of 600 mM sodium phosphate	7.2	24
Elution	Gradient elution from 150–600 mM (24% to 100%) sodium phosphate	7.2	11
Column wash	600 mM sodium phosphate	7.2	5

Table 3. Dengue purification protocol.

Step	Mobile Phase	pH	Volume, ml
Pre-wash	1 M NaOH		5
Wash	600 mM sodium phosphate	7.2	10
Equilibration	10 mM sodium phosphate	7.2	20
Sample loading and washing	10 mM sodium phosphate	7.2	20
Elution	Gradient elution from 10–600 mM sodium phosphate	7.2	15
Column wash	600 mM sodium phosphate	7.2	5

Viral Activity Assay

Influenza viral activity in the recovered fractions was determined via hemagglutination analysis (HA test) with chicken red blood cells. Activity of the dengue virus in the recovered fractions was determined via HA test with goose red blood cells as described previously (Kurosawa et al. 2012).

Estimating Double-Stranded DNA (dsDNA)

The concentrations of dsDNA in fractions were determined using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen Corporation) according to the manufacturer's instructions.

Protein Analysis in Virus Fractions

Viral fractions were analyzed for total protein concentrations with the Micro BCA Protein Assay Kit. Host cell protein (HCP) analysis for the influenza viral fractions was performed with the MDCK HCP ELISA Kit (Cygnus Technologies).

$TCID_{50}$ Influenza Virus Infectivity Assay

A confluent monolayer of MDCK cells in 96-well microplates was used for this test. MDCK cells (2 x 10^4 cells/well) were cultured in flu culture medium for 1 day prior to the testing. Each virus fraction underwent tenfold serial dilution with flu culture medium. Diluted viral fractions (50 µl) were added to each well (n = 3) and cultured for one week. Cytopathic effects were studied under a microscope. Titers were calculated using the Reed-Muench method.

Viral Purity Analysis

Viral purity was assessed by electrophoresis on a 12.5% SDS-PAGE gel and by immunoblotting. The protocols used for these analyses were as described previously (Kurosawa et al. 2012). Cell culture fluid was concentrated tenfold and all other samples were concentrated 30-fold by ultrafiltration with a 10 kD MWCO filter before use. Bio-Rad's Gel Doc EZ Imaging System was used to quantitate band density. For immunoblotting, primary antibody anti-HA mAb (Abnova) was used at a 1:200 dilution with the influenza virus samples and mouse anti-D2 serum (made in house by HOYA Technosurgical Corporation for these experiments) was used at a 1:500 dilution with the dengue virus samples. Secondary antibody anti-mouse IgG (Fc)-POD (American Qualex) was used at a 1:1,000 dilution. The bands were visualized with EzWestBlue (ATTO Corporation).

Results

Influenza Virus

Viral Activity

The chromatographic results from the influenza virus purification are shown in Figure 1. Influenza virus elutes at approximately 250 mM phosphate, with good separation from the bulk of UV-absorbing material and partial resolution from dsDNA. Hemagglutination activity (red bars on the chromatograms) measures the titer of virus that can bind to molecules present on the surface of red blood cells and cause agglutination. As seen in Figures 1A and 1B, HA activity is predominantly seen between retention volumes of 26 and 31 ml. The total viral activity recovered from these fractions was calculated to be about 75% of the starting activity. This activity was well separated from the bulk of the DNA and A280-absorbing impurities. The samples showing bulk viral activity were pooled for further analytical assays.

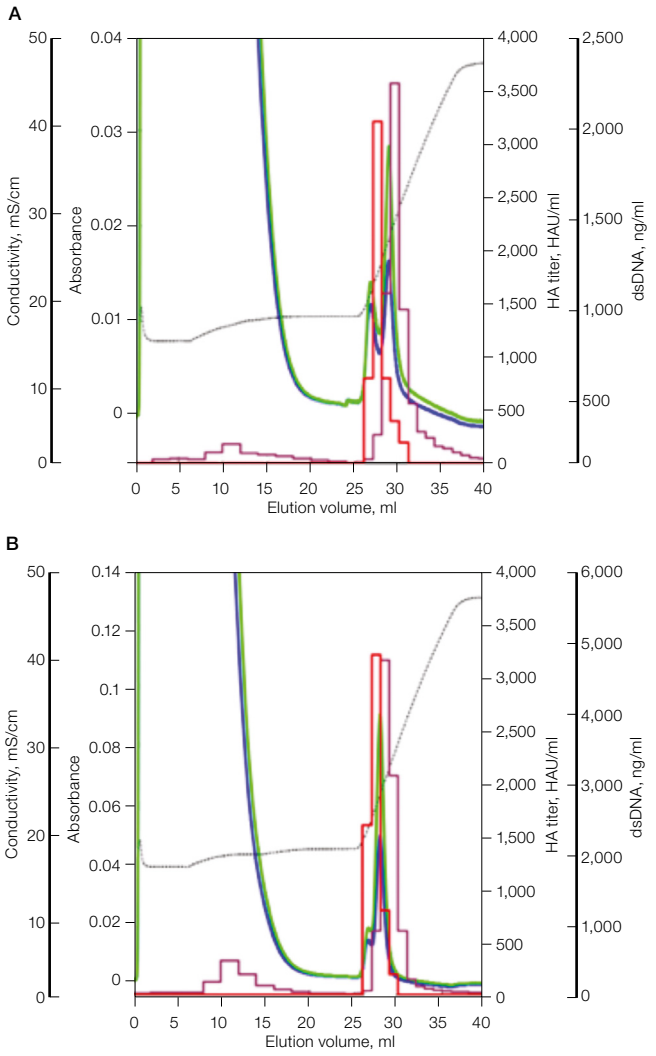

Fig. 1. Replicate elution of influenza virus from CHT XT.
HA titer (—); DNA (—); A_{280} (—); A_{260} (—); conductivity (—).

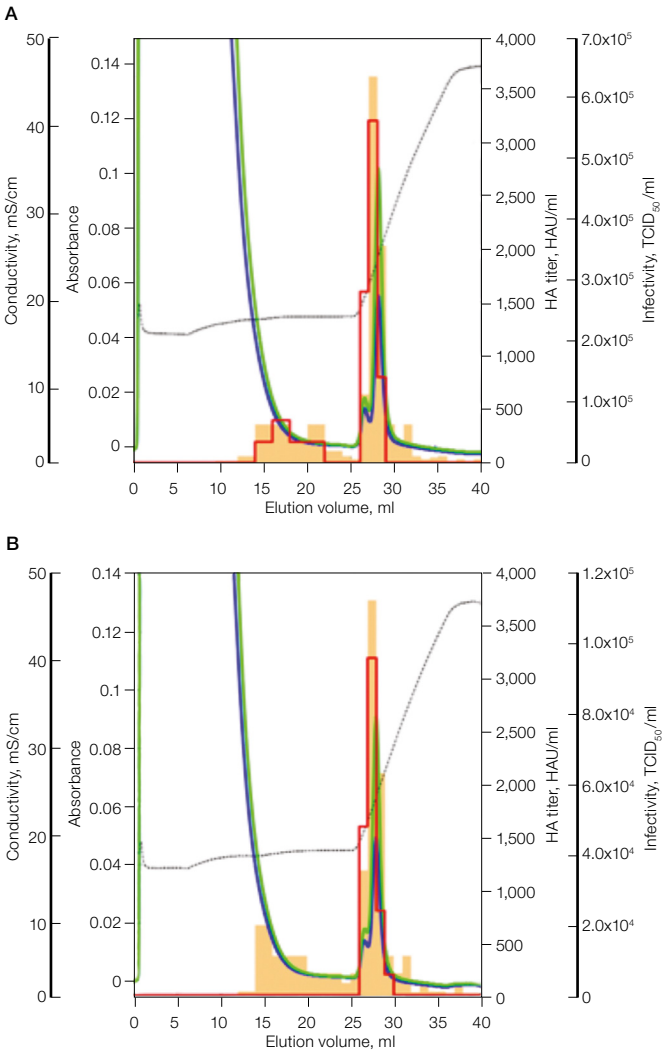

Fig. 2. Infectivity of the influenza virus purified on CHT XT.
Infectivity (—); HA titer (—); A_{280} (—); A_{260} (—); conductivity (—).

Viral Infectivity

In order to determine if the purified influenza virus maintained its infectivity, a $TCID_{50}$ test was performed (Figure 2) to assay for cytopathic effects (CPE). This test determines the quantity of active, infectious particles. A mean recovery of ~68% infectivity was calculated for the replicates. As seen in Figures 2A and 2B, the bulk of the infectivity is seen at retention volumes that also showed the highest HA activity. This confirmed that fractions 26 to 32 contained active, purified virus.

Analytical Assays

Three analytical assays were performed on the pooled samples from the influenza virus run on CHT XT Media. The results of the analytical assays are shown in Table 4. Single-step influenza virus purification on CHT XT led to significant (>99%) clearance of total and host cell proteins as well as removal of over 60% of dsDNA.

Table 4. Results from the analytical assays on the pooled influenza samples from the HA assay.

Log Reduction in dsDNA	Log Reduction in Total Protein	Log Reduction in HCP Content
0.42	2.82	2.54

Viral Purity

SDS-PAGE analysis of the active pooled fractions from Figure 1 confirmed the significant purification of species migrating at the expected molecular weight for the two forms of the HA protein (Figure 3A). Immunoblotting of the same samples confirmed that the concentrated bands are HA (Figure 3B).

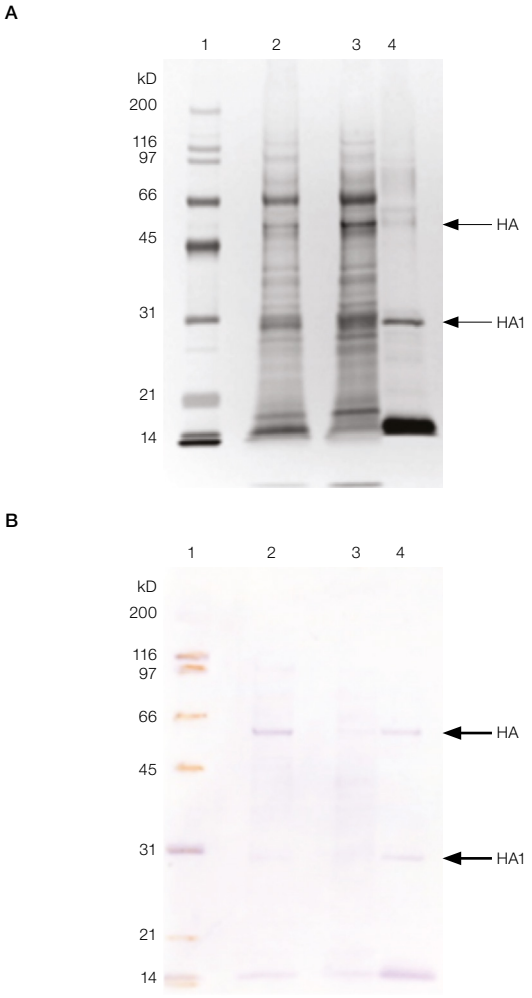

Fig. 3. SDS-PAGE (A) and immunoblotting analysis (B) of fractions.
Protein bands on the gels were visualized by silver staining and the HA proteins in active samples were detected using an HA-specific monoclonal antibody. Lane 1: 3 µl protein MW marker; lane 2: 10 µl cell culture fluid; lane 3: 10 µl flow-through fractions; lane 4: 10 µl pooled fractions from HA assay.

Dengue Virus

Viral Activity

The chromatographic results from the dengue virus purification are shown in Figure 4. Dengue virus elutes at approximately 400 mM phosphate, with good separation from the bulk of UV-absorbing material and dsDNA. HA activity was predominantly seen between retention volumes of 28 to 38 ml; fractions 30–35 were pooled for further analysis. The total viral activity recovered from these fractions was calculated to be about 87%. Viral activity was not detected in the flow-through fractions in any of the replicates.

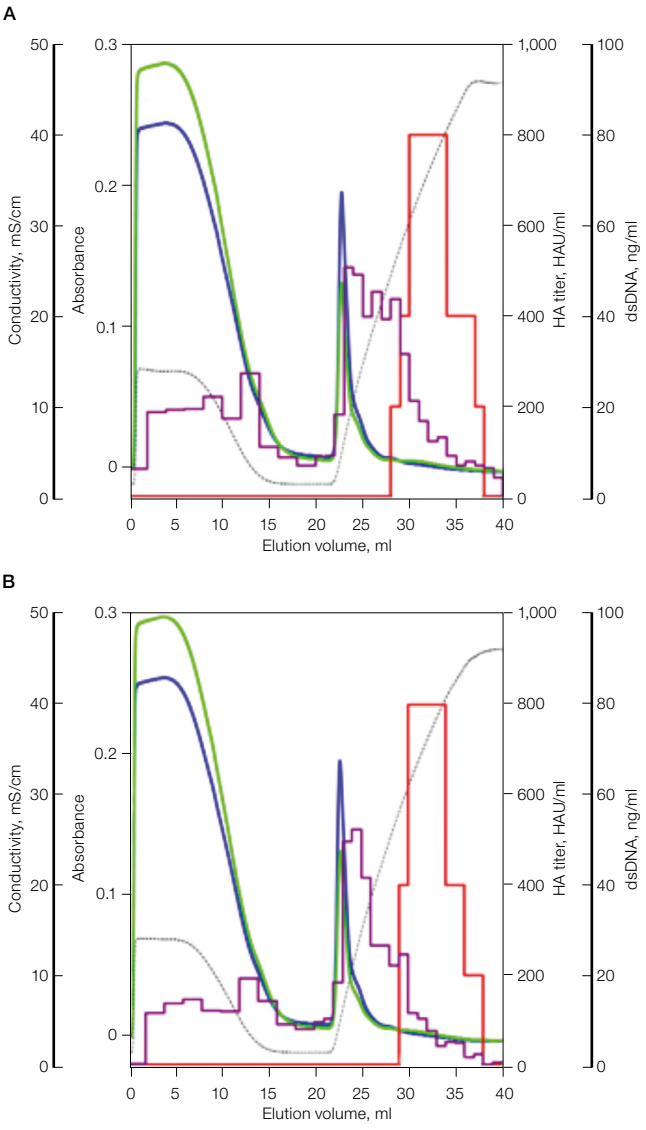

Fig. 4. Replicate elution of dengue virus from CHT XT.
HA titer (—); DNA (—); A_{280} (—); A_{260} (—); conductivity (—).

Analytical Assays

Two analytical assays were performed on the pooled samples from the dengue virus run on CHT XT. The results of the analytical assays are shown in Table 5. Single-step dengue virus purification on CHT XT led to significant clearance of protein (>93%) and dsDNA (>91%) contaminants.

Table 5. Results from the analytical assays on the pooled dengue samples from the HA assay.

Log Reduction in dsDNA	Log Reduction in Total Protein
1.07	1.15

Viral Purity

SDS-PAGE analysis of the pooled active retention fractions from Figure 4 showed that the pool contains proteins that migrate at the expected position for dengue E and C proteins as shown on the immunoblot (Figure 5). Note that a contaminant can be seen migrating immediately above the E protein on the SDS-PAGE gel.

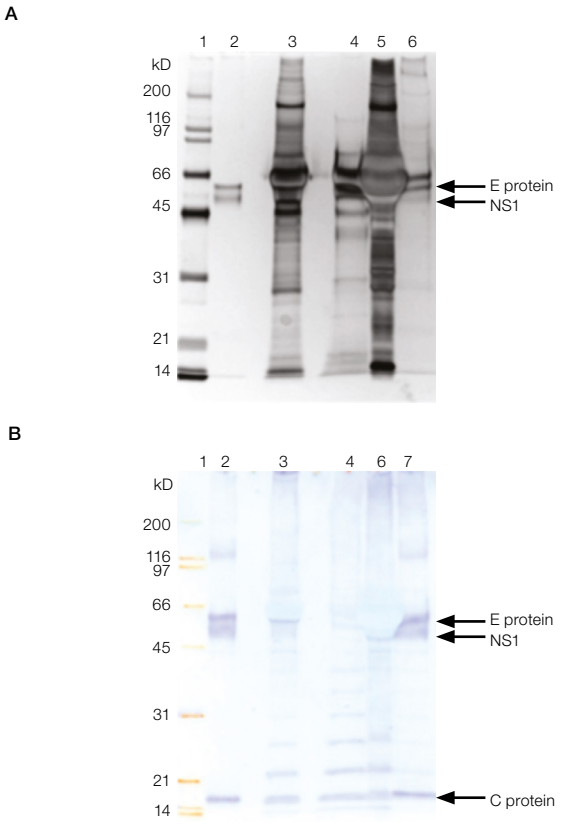

Fig. 5. SDS-PAGE (A) and immunoblotting analysis (B) of fractions. Protein bands on the gels were visualized by silver staining and the viral proteins in active samples were detected by antiserum against dengue virus type 2. Lane 1: 3 µl protein MW marker; lane 2: 5 µl dengue positive control; lane 3: cell culture fluid; lane 4: 10 µl flow-through fractions; lane 5: 5 µl UV-rich fractions; lane 6: 10 µl pooled fractions from HA assay.

Conclusion

Chromatography media are continuously evolving to meet the increasing demands of the bioprocessing industry. We developed the new CHT XT Media to meet the demand for a robust media with excellent life time and single-step purification functionality for healthy process economics. With both high purity and high yield for both influenza and dengue viruses, we show that CHT XT can be used for single-step impurity clearance. This positions CHT XT as a media for simple, fast, scalable, and reproducible viral purification.

References

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Moritz A (2005). Virus safety evaluation of biotechnology products in development. BioProcess Int Suppl. 3, 15–16.

High Dynamic Binding Capacity for Efficient Downstream Purification of Large Biomolecules

Introduction

Nuvia HP-Q is a high-performance strong anion exchange resin. It is the latest product in the Nuvia family of high-capacity ion exchange resins and can be used for downstream purification of large molecules such as high molecular weight (HMW) plasma proteins IgA and IgM, viruses, virus-like particles (VLPs), and PEGylated proteins. It is built on the rugged and hydrophilic UNOsphere epoxide base bead that provides the fast mass transfer kinetics and low nonspecific binding demanded by today's process manufacturing. The stability of this base bead and its broad chemical compatibility allows repeated uses with a long resin lifetime. Nuvia HP-Q particle size is designed to offer high dynamic binding capacity (DBC) at fast flow rates without excessive backpressure, thereby delivering excellent process economics. Its pore size is optimized for easy accessibility and adsorption of large biomolecules, and the internal spacer length and ligand density facilitate efficient binding of the biomolecules even at high flow rates.

Nuvia HP-Q was designed to overcome the issues faced when purifying large biomolecules with other commercially available resins and help in downstream purification of such biomolecules at fast flow rates without loss in DBC and recovery. IgM obtained from plasma fractionation showed DBC in the range of 20–25 mg/ml with Nuvia HP-Q. Table 1 shows that higher DBC at faster flow rates was obtained with Nuvia HP-Q relative to other resins from different vendors. The technical properties of Nuvia HP-Q Resin are listed in Table 2.

Table 1. Superior DBC of Nuvia HP-Q at a high flow rate relative to other commercially available resins.

Resin	Matrix Material	Particle Size, µm	Pressure, bar	Recommended Flow Rate, cm/hr[1]	DBC (IgM)
Nuvia HP-Q	**UNOsphere epoxide**	**50**	**<3**	**300**	**+++[2]**
Resin 1	Dextran beads	50	<3	30	+++[2]
Resin 2	Agarose	75	<3	300	+[3]
Resin 3	PS/DVB	50	<3	300	+[2]
Resin 4	PMMA	50	<3	300	+[2]

[1] Recommended flow rate for industrial scale column (D > 30 cm).
[2] DBC data at 10% breakthrough.
[3] Data obtained from vendor presentation.

Table 2. Properties of Nuvia HP-Q.

Property	Description
Type of ion exchanger	Strong anion
Functional group	$-N^{+}(CH_3)_3$
Particle size range	38–53 µm
Total ionic capacity	48–88 µeq
Dynamic binding capacity*	>50 mg/ml at 100 cm/hr
Recommended linear flow rate	50–300 cm/hr
Maximum operating pressure	3 bar
Compression factor	~1.2
Long-term pH stability	2–11
Shipping solution	20% ethanol or 2% benzyl alcohol
Regeneration	1–2 M NaCl
Sanitization	1 N NaOH
Storage conditions	20% ethanol or 0.01 N NaOH
Storage temperature	Room temperature
Chemical stability	1 N NaOH (20°C), up to 1 week
Shelf life	5 years

* 10% breakthrough capacity determined with 1.1 mg/ml of thyroglobulin in 20 mM Tris HCl, pH 8.0.

Best-in-Class Binding Capacity

Nuvia HP-Q delivers on the demanding pressure flow requirements of downstream process purification. Its optimized particle size facilitates a high DBC at high flow rates (Figure 1). Parallel runs of thyroglobulin on Nuvia HP-Q and other commercially available resins demonstrate that Nuvia HP-Q overcomes the compromised productivity issue that results from low DBCs at process-scale flow rates (Figure 2).

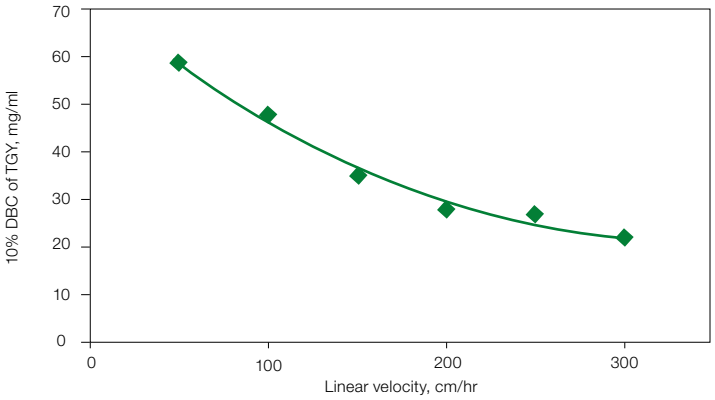

Fig. 1. Dynamic binding capacity (DBC) vs. flow velocity of Nuvia HP-Q. The resin was packed into a 1 ml Bio-Scale Mini Column (0.56 x 4 cm). Thyroglobulin (TGY) solution (1.1 mg/ml) in 20 mM Tris Cl, pH 8.0 was loaded onto the column until 10% breakthrough was observed.

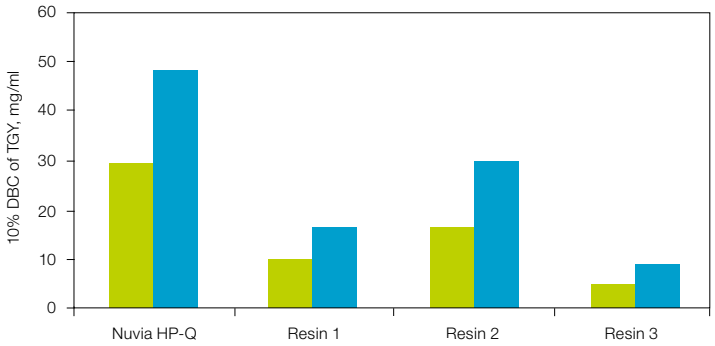

Fig. 2. Dynamic binding capacity (DBC) vs. residence time of Nuvia HP-Q. Comparison of DBCs between Nuvia HP-Q and three other commercially available resins at 1.2 and 2.4 min residence times. The resins were packed into 1 ml columns. Thyroglobulin (TGY) solution (1.1 mg/ml) in 20 mM Tris Cl, pH 8.0 was loaded onto the columns until 10% breakthrough was observed. 1.2 min residence (■); 2.4 min residence (■).

Excellent Pressure Flow Properties

Nuvia HP-Q Resin is designed with an optimal bead size to achieve both laboratory- and process-scale purification of large biomolecules at high flow rates without being limited by column pressure. This leads to an increase in productivity during protein purification. The column pressure remains below 1.5 bar at a linear velocity of 350 cm/hr (Figure 3).

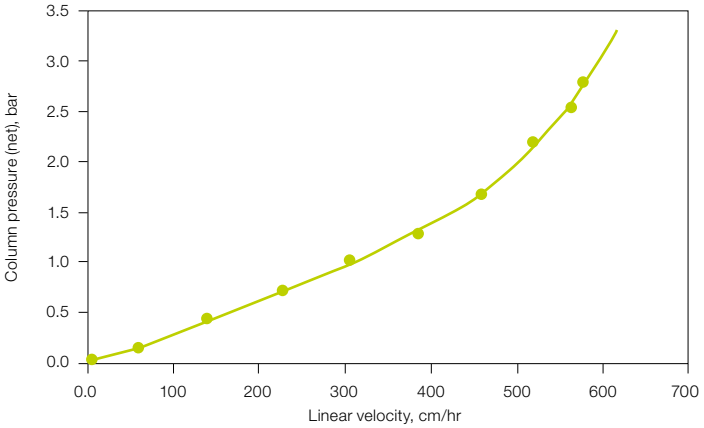

Fig. 3. Pressure/flow performance of Nuvia HP-Q Resin. Nuvia HP-Q slurry prepared in 1x PBS, pH 7.5 was packed into a 20 x 20 cm column by axial compression with a compression factor of 1.2.

Robust Performance and Recovery

Nuvia HP-Q Resin is produced by a validated manufacturing process that ensures batch-to-batch reproducibility. The chemical stability of Nuvia HP-Q allows the resin to perform consistently with minimal changes to DBC or recovery even after prolonged exposure to NaOH (Figure 4).

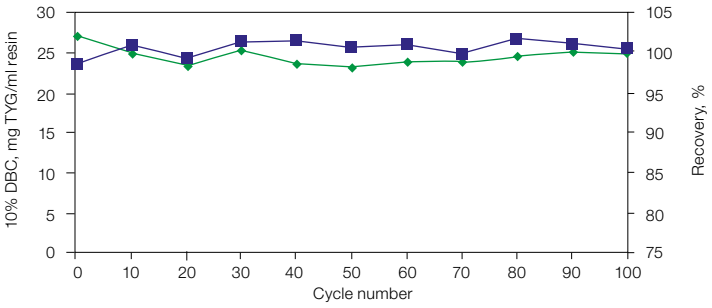

Fig. 4. Stability, reusability, and recovery with Nuvia HP-Q Resin. Thyroglobulin (TYG) solution (1.1 mg/ml) in equilibration buffer (20 mM Tris Cl, pH 8.0) was loaded onto a 1 ml Bio-Scale Mini Column (0.56 x 4 cm) packed with Nuvia HP-Q to a compression factor of 1.2. The column was operated at 300 cm/hr. The protein was eluted in 5 CV of elution buffer (20 mM Tris Cl, 1 M NaCl, pH 8.0) at 300 cm/hr. Cleaning in place (CIP) was performed on the column with 3 CV of 0.5 N NaOH at 300 cm/hr followed by a 40 min hold. The 10% DBC at linear velocity of 300 cm/hr was determined after every 10 cycles. DBC (◆); recovery (■).

Easy Scalability from Laboratory to Bioprocess Manufacturing

Nuvia HP-Q Resin is specifically designed for easy scalability to meet manufacturing demands. It is available in multiple user-friendly formats, including prepacked Foresight Columns and Plates for purification condition screening and bulk bottles for pilot- to manufacturing-scale purifications. It is backed by our regulatory support documentation and security of supply commitment.

Practical Guide: Selecting the Optimal Resins for Adenovirus Process Purification

Purification Solutions

On the Quest to Purify a Large Complex Virus

The number of gene therapy–based treatments has grown significantly since they first appeared nearly three decades ago. This has created profound optimism about our potential to develop a cure for diseases such as cancer and AIDS. One of the most effective contributors to the success of gene therapy is the ability to use viruses as vehicles for delivering genes to their targets. Initially, murine retroviruses were recruited for this purpose, but more recently, adenovirus (Ad) and adeno-associated virus (AAV) have become the vehicles of choice. In fact, Ad and AAV studies account for over 25% of all ongoing gene therapy trials. However, producing sufficient quantities of pure clinical-grade virus, which is required to ensure biosafety, is not an easy task.

One of the main barriers to achieving high purity levels is the size and complexity of the adenovirus. One intact virus particle contains more than 2,700 protein subunits, has a mass of ~165 MDa, and has a diameter of ~0.1 µm. This complexity renders traditional virus purification methods such as filtration, density gradients, and ultracentrifugation inefficient. In addition, the virus has thousands of charge variants, making it difficult to establish well-defined binding and elution conditions. Therefore, purification protocols and strategies used for small and mid-sized viruses also fall short when it comes to adenoviruses. Furthermore, adenoviruses can be acid-labile, which further increases purification challenges. These hurdles call for alternate strategies for achieving efficient adenoviral purification. In response, column chromatography has gained popularity over the past two decades as a way to overcome the challenges of process adenoviral purification and the limitations of traditional purification methods (Huyghe et al. 1995).

Bio-Rad has provided a progressive selection of chromatography resins for process-scale purification of viruses for more than 50 years. After screening five different chromatography resins, we developed a two-column capture and polish cGMP-ready purification strategy for a recombinant adenovirus. We show that this process yields an active concentrated product with purity, host cell protein (HCP), and DNA contamination levels comparable to other clinical-grade products. Additionally, the process is readily scalable and sufficiently simple, rapid, and efficient for the production of clinical-grade viral vectors for gene therapy–based treatments. This guide provides a brief snapshot of the various resins considered, the reasons behind the selection of the final two resins, and the results from our study.

Design of Experiment (DoE) for the Process Purification of Adenoviruses

Initial Screening with Five Bio-Rad Resins

Four ion exchange (IEX) and one mixed-mode (MM) resins were initially screened to determine which had the potential to be used for mass capture of adenoviruses. As shown in Table 1, use of two cation exchange (CEX) resins, UNOsphere S and Nuvia S, left the majority of the virus in the flowthrough and/or wash samples. This makes them unsuitable for both bind-and-elute and flow-through modalities relative to the three other resins — Nuvia cPrime, UNOsphere Q, and Nuvia Q — whose use resulted in the majority of the virus remaining in the eluate. Nuvia cPrime is a MM resin that offers a unique balance between hydrophobic and charged characteristics. It is built on a mechanically and chemically stable, rigid, macroporous base matrix with particle size optimized to provide exceptional flow properties, fast mass transfer, and stability (bulletin 6242). Both Nuvia Q and UNOsphere Q are anion exchange (AEX) resins. Nuvia Q Resin, with its high binding capacity, delivers excellent performance for polishing applications. It can significantly improve productivity while contributing to reduced capital costs, space requirements, and cycle time for downstream purification (bulletin 6129).

UNOsphere Q Resin has large-diameter pores and a large surface area to maximize capture speed, macromolecule capacity, recovery, and productivity (bulletin 2724). It shows a high binding capacity of 125–180 mg/ml bovine serum albumin (BSA) at a flow rate of 150–1,200 cm/hr.

Table 1. Results from the initial resin screening.

Column type	Virus in flowthrough/ wash	Virus in eluate	Notes/Implications
UNOsphere S (CEX)	+++	++	Poorly suited for virus purification in both bind-and-elute and flow-through modalities
Nuvia S (CEX)	+++	++	Poorly suited for virus purification in both bind-and-elute and flow-through modalities
Nuvia cPrime (MM)	—	++++	Partial elution in 125 mM NaCl, pH 6.5; hence, dilution of crude harvest required prior to column loading
UNOsphere Q (AEX)	—	++++	Could be considered for direct mass capture
Nuvia Q (AEX)	—	++++	Could be considered for direct mass capture

Selection of the Mass Capture Resin

Of the three resins with potential for use in the mass capture process, Nuvia cPrime was selected for the following reasons. The main anticipated impurity in the feed is serum albumin. Nuvia Q and UNOsphere Q bind to albumin, decreasing the effective binding capacity for the virus. Nuvia Q also binds host cell DNA and other negatively charged impurities like lipopolysaccharides (LPS). Therefore, they were not good candidates for the mass capture step. On the other hand, albumin and the negatively charged impurities come out in the flowthrough with Nuvia cPrime. In addition, the use of Nuvia cPrime requires a smaller column and decreases the potential for column fouling. Hence, Nuvia cPrime was better suited for the mass capture step. A representative chromatogram from this mass capture is shown in Figure 1.

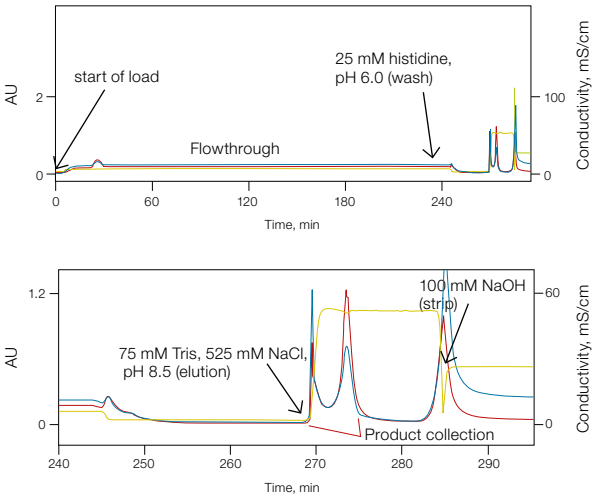

Fig. 1. Representative chromatogram from the Nuvia cPrime mass capture step. OD 260 (—); OD 280 (—); conductivity (—). AU, absorbance units.

Selection of the Polish Resin

The eluate from the Nuvia cPrime capture step had an NaCl concentration of ~500 mM. This rendered the Nuvia Q Resin more suitable for the polish purification step because it is able to adsorb virus at high NaCl concentrations. A representative chromatogram from this polish step is shown in Figure 2.

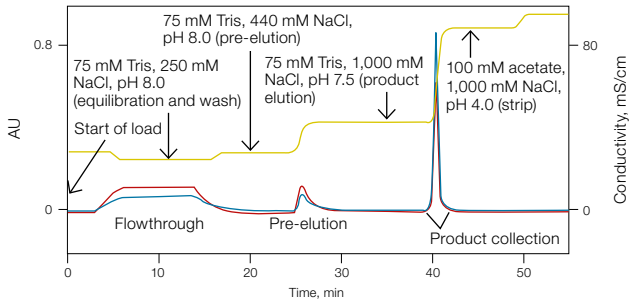

Fig. 2. Representative chromatogram from the Nuvia Q polish purification step. OD 260 (—); OD 280 (—); conductivity (—). AU, absorbance units.

Results

The initial capture purification of the recombinant adenovirus with Nuvia cPrime achieved a tenfold reduction in the processing volume and a significant reduction in feedstream contaminants (Figure 3, lanes 2–4). The final polish purification step with Nuvia Q achieved an additional twofold reduction of product volume along with a significant improvement in product purity (Figure 3, lanes 4–7). The five most prominent viral proteins, hexon, penton, core (V), hexon (VI), and core (VII) are readily visible in the final purified product (Figure 3, lane 7), whereas nonviral proteins are essentially absent.

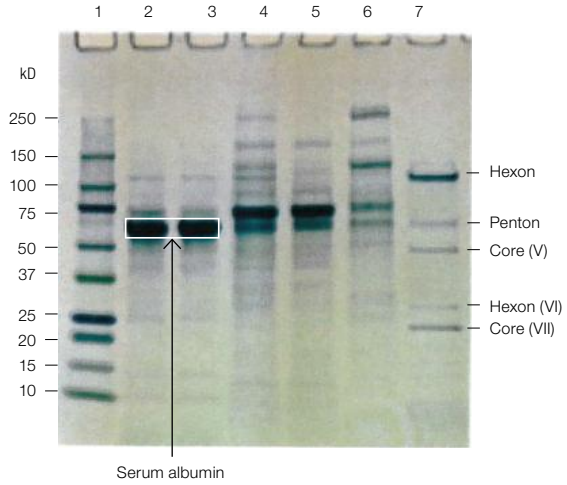

Fig. 3. SDS-PAGE analysis of the intermediates and the final product. Lane 1, MW marker; lane 2, Nuvia cPrime load; lane 3, Nuvia cPrime flowthrough; lane 4, Nuvia cPrime elution/Nuvia Q load; lane 5, Nuvia Q flowthrough; lane 6, Nuvia Q pre-elution; lane 7, final product.

This process yields an active, concentrated virus product with purity, HCP, and DNA levels comparable to clinical-grade products (Table 2). In addition, this protocol is less laborious and time consuming than other purification methods.

Table 2. Viral particle recovery and impurity clearance.

Sample	Total virus ($x10^{11}$ particles)	Impurity levels ($ng/10^{10}$ particles)	
		DNA	HCP
Bulk harvest	30.6	3,144	n/d
Nuclease-treated harvest	31.8	30	3,022
Nuvia cPrime eluate	18.4	n/d	58
Nuvia Q eluate	16.4	<0.02	2

n/d, not determined.

The resins and conditions used for each step in your purification process will have to be optimized based on your adenovirus feed. If you are interested in purifying small to mid-sized viruses, such as dengue virus, poliovirus, or Japanese encephalitis virus, a different mixed-mode media — CHT Ceramic Hydroxyapatite — would be the ideal choice to begin with. The details of such virus purifications are described in bulletin 6790 and bulletin 6549.

The information provided here can help you get started on your adenovirus purification strategy. For technical/product support or to request a quote, email your regional Bio-Rad representative at process@bio-rad.com or contact our customer service at 1-800-4-BIORAD (1-800-424-6723).

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Mammalian Virus Purification Using Ceramic Hydroxyapatite

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Process Separations

Introduction

Viruses can infect mammalian cells and cause diseases such as influenza, hepatitis, yellow fever, smallpox, and AIDS. Since some biotherapeutic products are produced using mammalian cell lines or plasma, the risk of viral contamination in these products is a concern and guidelines have been enforced to alleviate this risk. Chromatographic separation of viral particles from process intermediates is a key part of ensuring viral safety in biotherapeutics (ICH Expert Working Group 1999, Möritz 2005). Additionally, purification of viral particles is used extensively in the study and characterization of these infectious agents. Understanding aspects of a virus, such as how it infects host cells, uses the host cells for reproduction, and evades the host immune system, aids scientists in determining how to use viruses for research and therapy.

In order to study a virus, a pure, high-quality, infectious population is required. Conventional techniques for mammalian virus purification, for uses such as vaccine production or biological studies, can produce material of variable quality and quantity, often with significant loss of particle infectivity.

In this paper, we report the use of ceramic hydroxyapatite media for purification of a wide variety of mammalian viruses. Chromatography using ceramic hydroxyapatite media is simple, easily scalable, and results in a concentrated preparation of highly active virus.

Materials and Methods

The viral particles used in this study are shown in Table 1.

Table 1. Viral type and size.

Virus	Family	Genus	Genome	Envelope	Size, nm
Dengue	Flaviviridae	Flavivirus	ssRNA	+	50
Japanese encephalitis	Flaviviridae	Flavivirus	ssRNA	+	50
Influenza	Orthomyxoviridae	Influenzavirus	ssRNA	+	80–120
Mouse hepatitis	Coronaviridae	Coronavirus	ssRNA	+	100–150
Adenovirus	Adenoviridae	Mastadenovirus	dsDNA	–	90
Poliovirus	Picornaviridae	Enterovirus	ssRNA	–	30
Feline calicivirus	Caliciviridae	Vesivirus	ssRNA	–	30–38

Viral Activity Assays

Viral activity was determined using the assays shown in Table 2. Protein contaminants were detected by UV absorbance at 280 nm and SDS-PAGE analysis. DNA derived from host cells was quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies Corporation, USA).

Table 2. Detection methods used for viral activity.

Detection Method	Virus
Hemagglutination (HA) test	Dengue, influenza, adenovirus
Plaque assay	Japanese encephalitis
50% tissue culture infective dose ($TCID_{50}$)	Poliovirus, feline calicivirus, mouse hepatitis

Standard Chromatography Protocol

Chromatography was performed using Bio-Rad's BioLogic DuoFlow System. Columns (4.6 x 35 mm, Sugiyama Shoji Co., Ltd., Japan) with a 10-µm frit were packed with 40-µm CHT Ceramic Hydroxyapatite Type II Media. Frit pore size was important, as smaller porosities significantly reduced virus recovery (Y. Kurosawa, unpublished data). The flow rate was 1 ml/min. The purification protocol is outlined in Table 3, unless otherwise noted.

Table 3. Standard purification protocol.

Step	Mobile Phase	pH	Volume, ml
Wash	600 mM sodium phosphate	7.2	5
Equilibration	10 mM sodium phosphate	7.2	10
Sample loading	10 mM sodium phosphate	7.2	10
Wash	10 mM sodium phosphate	7.2	10
Elution	Gradient elution from 10–600 mM sodium phosphate	7.2	15
Wash	600 mM sodium phosphate	7.2	5

Results

Dengue Virus

Figure 1A shows the recovery of dengue virus type 2 from cell culture fluid. HA activity was recovered near the end of the gradient, separated from the bulk of A_{280}-absorbing material and from dsDNA (Kurosawa et al. 2012b). Figure 1B demonstrates that decreasing the flow rate by tenfold improves the sharpness of the elution peaks and, hence, separation. In both cases, recovery of HA activity was greater than 95%. Recent studies have indicated that adsorption of dengue virus particles to the surface of CHT Type II Media is similar to their adsorption to cells (Saito et al. 2013).

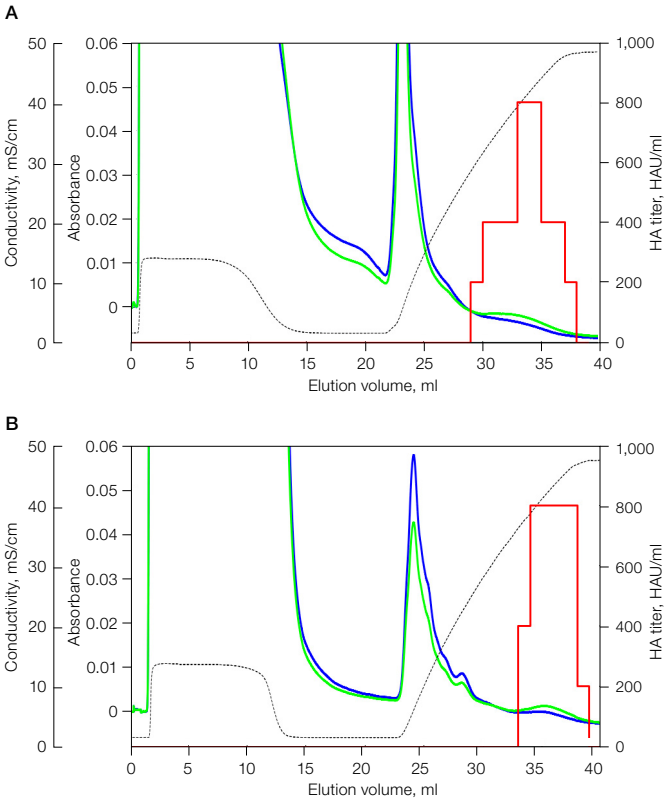

Fig. 1. Chromatograms of the separation of dengue virus type 2 by CHT Type II Media. A, flow rate at 1.0 ml/min; B, flow rate at 0.1 ml/min. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (- -); viral activity in HA test (—).

Other serotypes of dengue virus also bound to and eluted from CHT Type II Media. The approximate elution points in the sodium phosphate gradient for each serotype are shown in Table 4. Types 2 and 4 eluted at roughly the same position.

Table 4. Elution points of dengue serotypes in a sodium phosphate gradient.

Virus Serotype	Approximate Elution Point, mM
1	250
2	450
4	425

Influenza Virus

Chromatography of influenza virus A/Beijing/262/95 and A/Panama/2007/99 (Schickli et al. 2001) cultured in the presence of 0.02% and 0.20% BSA, respectively, is shown in Figure 2.

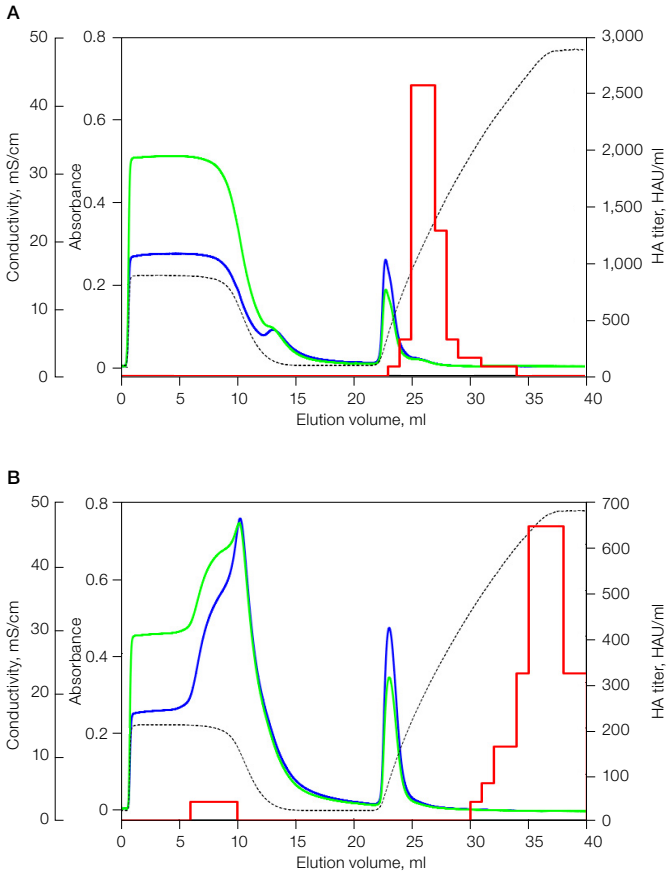

Fig. 2. Chromatography of influenza virus. A, A/Beijing/262/95; **B**, A/Panama/2007/99. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (**—**); viral activity in HA test (—).

HA activity is separated from a small BSA peak and a significant amount of material that did not bind to the column. Recovery, as measured by the HA assay, was 98% for the A/Beijing/262/95 virus. Higher concentrations of sodium phosphate are required to elute the A/Panama/2007/99 virus. In addition, the retention time was not affected by the source (allantoic fluid vs. cell culture; data not shown).

Mouse Hepatitis Virus

Mouse hepatitis virus (MHV) is a coronavirus (CoV), a genus that includes SARS-CoV. Two strains of MHV (MHV-NuU and MHV-S) (Hirano et al. 1981) were applied and bound to CHT Type II Media. Both were eluted at 26–28 minutes in the gradient (Figure 3).

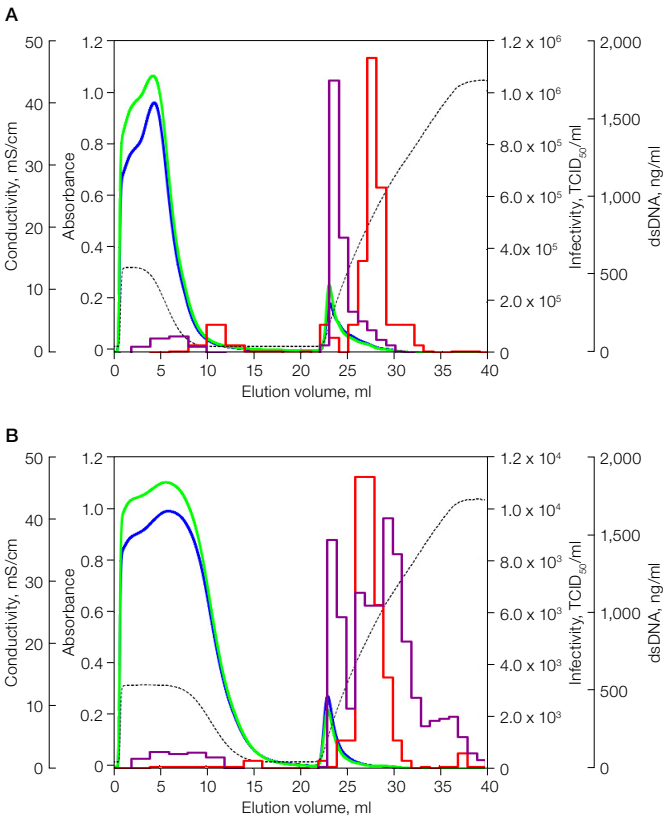

Fig. 3. Chromatography of two strains of MHV (A, MHV-NuU; B, MHV-S). UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (**- -**); viral infectivity in $TCID_{50}$ (—); dsDNA (—). Culture fluid contained 10% fetal bovine serum (FBS).

Nonenveloped Viral Particles

Nonenveloped viral particles can be purified by ceramic hydroxyapatite chromatography in the same way as enveloped viruses. Adenovirus (AdV) type 27, feline calicivirus (FCV) A391 (Hirano et al. 1986), and poliovirus (PV) Sabin type 2 all adsorbed to CHT Type II Media (Figure 4), although they showed different elution times.

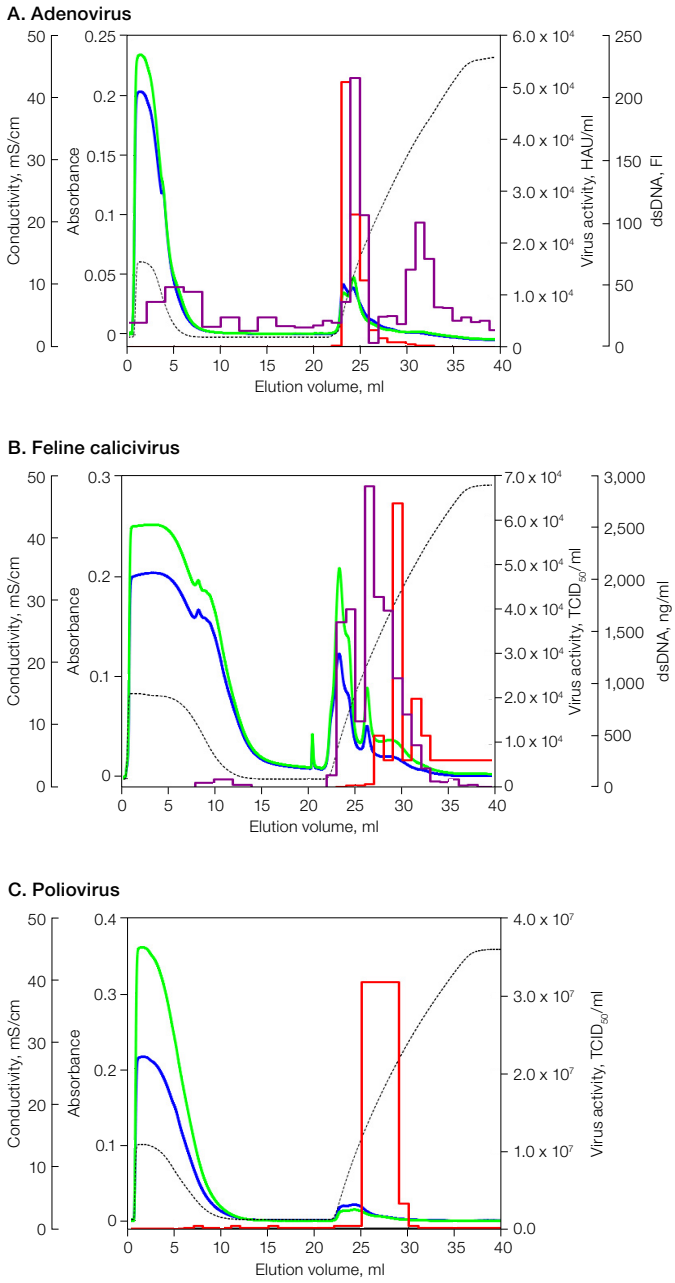

Fig. 4. Chromatograms of the separation of cell lysate (A) or culture fluid (B, C) containing nonenveloped viral particles by CHT Ceramic Hydroxyapatite Type II Media. A, AdV type 27; **B**, FCV A391; **C**, PV Sabin type 2. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (—); viral activity (AdV in HA test, FCV and PV in $TCID_{50}$) (—); dsDNA (—). Cell culture fluid contained 10% FBS. FI, fluorescence intensity.

Japanese Encephalitis Virus

Japanese encephalitis virus (JEV) chromatography is shown in Figure 5 (Kurosawa et al. 2009, 2012a). Irrespective of the source or strain, the virus elutes at approximately 350 mM sodium phosphate (note that the gradient in these two cases is 10–400 mM and the column size is 6.8 x 20 mm). Again, there is good separation between protein contamination and the virus.

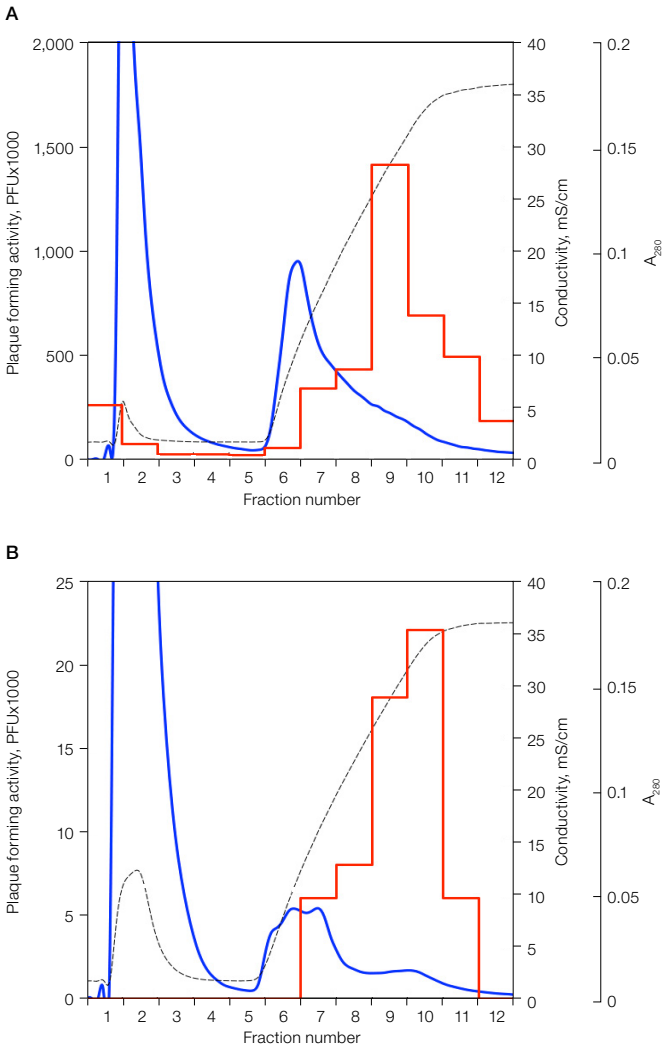

Fig. 5. JEV chromatography at pH 7.0. A, mouse brain homogenate infected with JEV JaGAr01; **B**, cell culture fluid of JEV Beijing. UV absorbance at 280 nm (—); conductivity (- -); infectious activity in plaque assay (—).

Note: Figure 5A is modified from Kurosawa et al. 2012a.

Effect of Hydroxyapatite Type on Separation

Figure 6 shows the separation of dengue virus type 2 from cell culture contaminants on four apatites: CHT Type I, CHT Type II, CFT Ceramic Fluorapatite Type II, and MPC Ceramic Hydroxyfluoroapatite Media. Yields were 80% or higher for each media type except for MPC, where the yield was 50%. Although binding and elution was achieved on all four media, the separation of virus from impurities was best on CHT Type II Media. Figure 7 shows a similar study using CHT Type II and CFT Type II Media for the purification of poliovirus, with recoveries of 88% and 102%, respectively. These results illustrate the importance of choosing the appropriate media for the separation in question.

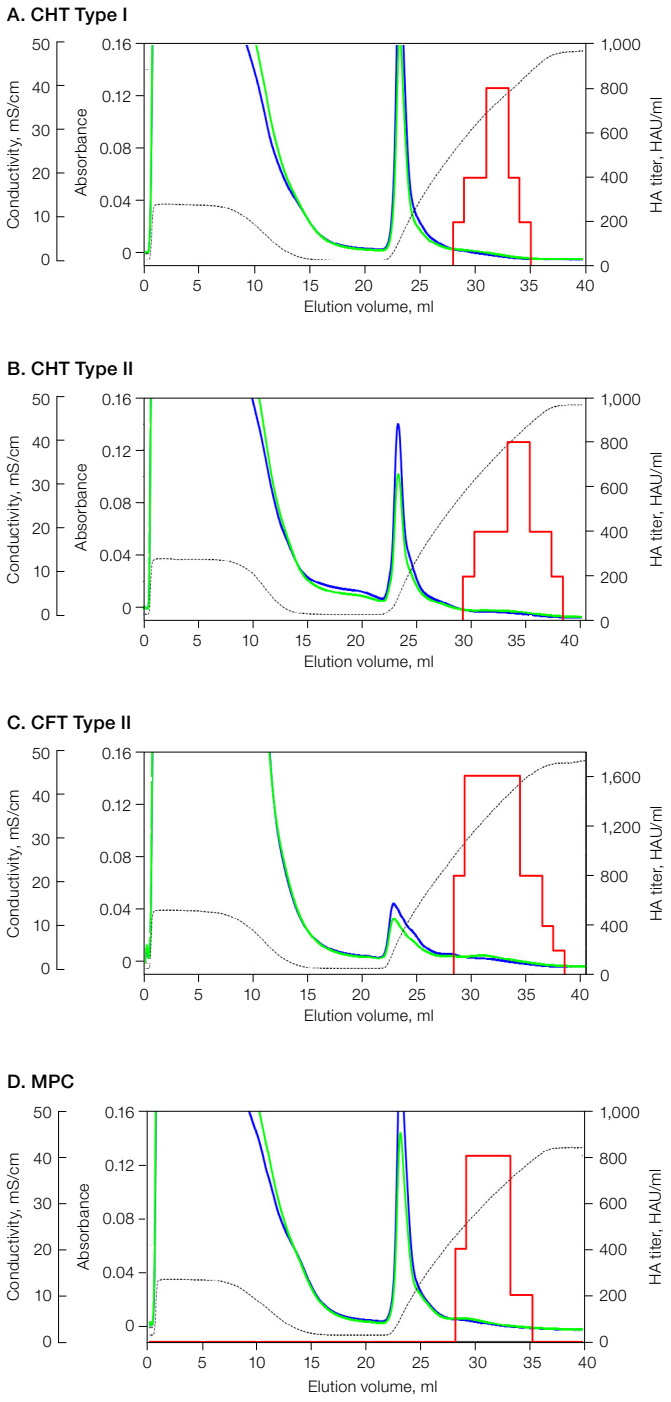

Fig. 6. Chromatograms of dengue virus type 2. A, CHT Type I Media; **B**, CHT Type II Media; **C**, CFT Type II Media; D, MPC Media. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (**--**); viral activity in HA test (—).

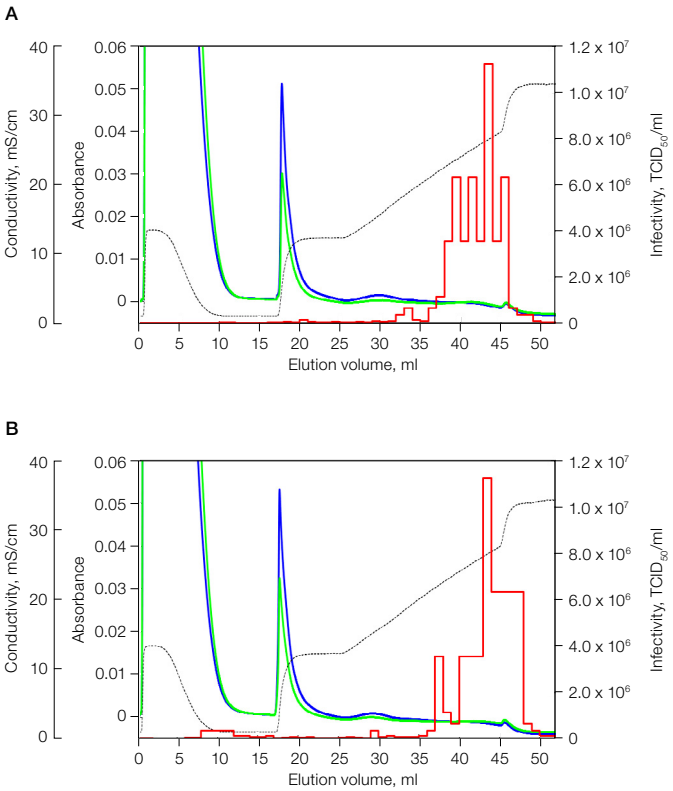

Fig. 7. Chromatograms of culture fluid of polio virus at pH 6.4. A, CHT Type II Media; **B**, CFT Type II Media. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity of elution buffer (**--**); infectious activity in $TCID_{50}$ (—). Note: the gradient in these two cases is 150–450 mM at pH 6.4 for 20 ml.

Conclusion

Using ceramic hydroxyapatite media provided high purity, recovery, and viral activity for seven mammalian viruses of varying size and belonging to different families. We have shown that, in at least one case, slowing the flow rate and decreasing the gradient slope allowed better purification of viral particles on CHT Type II Media, signifying the importance of determining the best settings for such factors when using apatite media. Testing different apatites is significant for determining which media type will work best for a specific virus. A larger pore size, as provided by the CHT Type II Media, allowed better separation of the dengue virus from contaminants, compared to other apatite media.

Of equal significance, the use of ceramic hydroxyapatite media is simple and provides reproducible results, allowing an alternative to the conventional methods of viral purification.

Acknowledgements

- Dengue viral particles were supplied by Professor Koichi Morita of the Department of Virology, Institute of Tropical Medicine, Nagasaki University, Japan.
- Experiments using influenza virus A/Beijing/262/95 and A/Panama/2007/99 were conducted in collaboration with Dr. Shota Nakamura and Dr. Takaaki Nakaya of Osaka University, Japan.
- The mouse hepatitis virus and feline calicivirus were supplied by Dr. Norio Hirano and Dr. Shigehiro Sato, and the poliovirus by Dr. Shigehiro Sato, of the Department of Microbiology, Iwate Medical University, Japan.

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New Selectivity and Large Design Space for Downstream Purification Processes

Introduction

Nuvia cPrime hydrophobic cation exchange media are a new addition to Bio-Rad's expanding family of mixed-mode purification products. Nuvia cPrime provides unique selectivity, high recovery, and versatility in large-scale purification applications for a variety of therapeutic proteins. Nuvia cPrime is effective for initial capture and polish applications, especially for molecules that present a purification challenge using current schemes.

Mixed-Mode Ligand

Nuvia cPrime media are designed with a mixed-mode ligand (Figure 1) that provides a unique balance between hydrophobic and charged characteristics. The ligand structure also provides an opportunity for hydrogen-bonding interactions. Importantly, the balance of weak acid and hydrophobic components is optimized to allow for straightforward method development and predictable behavior during binding and elution.

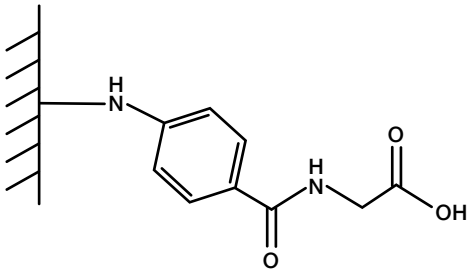


Fig. 1. Mixed-mode ligand for Nuvia cPrime media.

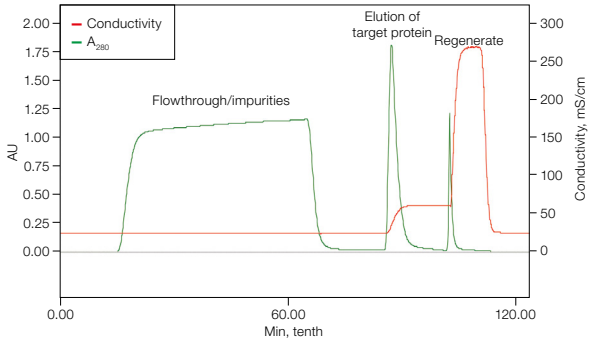
Properties of Nuvia cPrime Media

Property	Description
Functional group	Hydrophobic weak cation exchange
Base matrix composition	Macroporous highly crosslinked polymer
Particle size	70 µm ± 10 µm
Dynamic binding capacity* (hIgG)	>40 mg/ml
Dynamic binding capacity (lactoferrin)	>60 mg/ml
Ligand density	123± 20 µeq/ml
Recommended linear flow rate	50–600 cm/hr
Pressure vs. flow performance**	Under 2 bar at a flow rate of 600 cm/hr
pH stability	
Short-term	3–14
Long-term	4–13
Chemical stability	8 M urea, 6 M GuHCl, 6 M KSCN, 3 M NaCl, 1% Triton X-100, 2% SDS + 0.25 M NaCl, 20% EtOH, 70% EtOH, 30% IPA
Shipping solution	20% ethanol, 30 mM Na_2SO_3
Storage conditions	0.1 N NaOH
Shelf life*	5 years

* At 10% breakthrough, 300 cm/hr.
** 20 × 20 cm packed bed (1.17 compression factor).

New and Unique Selectivity

The balance between hydrophobicity, charged interaction, and the highly hydrophilic base matrix of Nuvia cPrime empowers method developers with new ways to directly exploit various modes of interaction to purify challenging or sensitive proteins or to separate closely related protein species, such as isoforms and variants from post-translational modifications, product aggregation, and degradation (Figure 2).



Chromatographic

Condition	Specification
Column	0.56 x 4 cm
Flow rate	300 cm/hr
Loading buffer	50 mM sodium phosphate + 150 mM NaCl (pH 6.5)
Elution buffer	50 mM sodium phosphate + 400 mM NaCl (pH 7.0)
Equilibration	Loading buffer
Wash	Loading buffer
Elution	Elution buffer
Regenerate	1 N NaOH

Fig. 2. Chromatographic performance and unique selectivity. Nuvia cPrime media enable effective separation. AU, absorbance units.

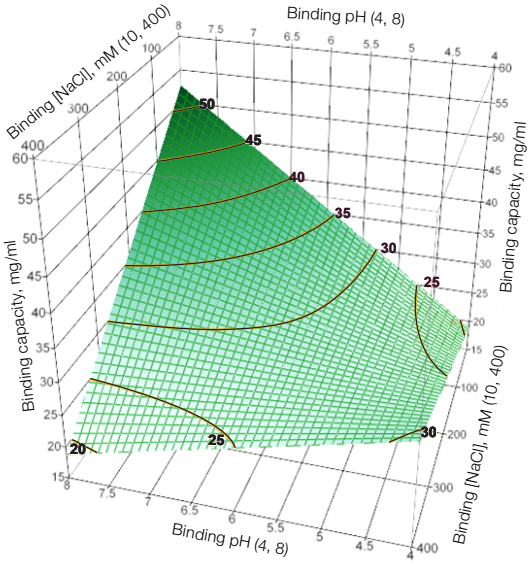
Large Design Space for Binding and Elution

Nuvia cPrime is designed for versatile capture and high recovery across a wide range of salt concentrations and pH (Figure 3). These properties may allow for direct loading without the need for dilution. Integrating a Nuvia cPrime step into a multicolumn process is operationally simple.

Chromatographic Performance for Novel Therapeutics

Nuvia cPrime is effective for the purification of established therapeutic proteins as well as the increasingly diverse new constructs that are in development (many of which lack an affinity handle). Salt- and pH-sensitive proteins with a high propensity for aggregation and/or degradation can now be effectively purified using simplified methods.

Lysozyme binding capacity, mg/ml



Lysozyme recovery, %

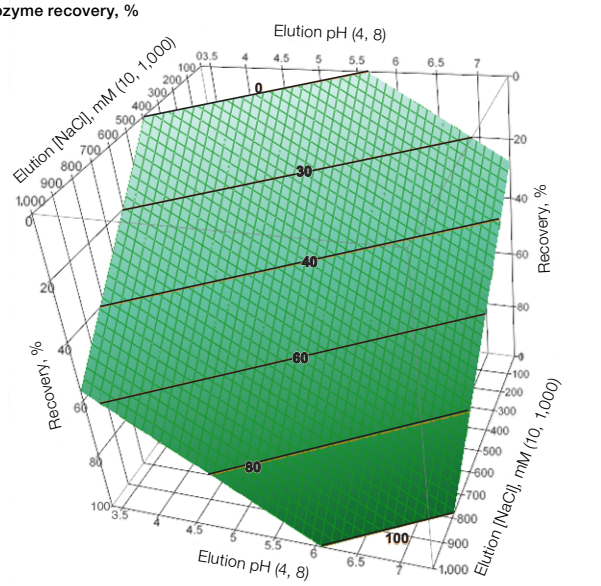


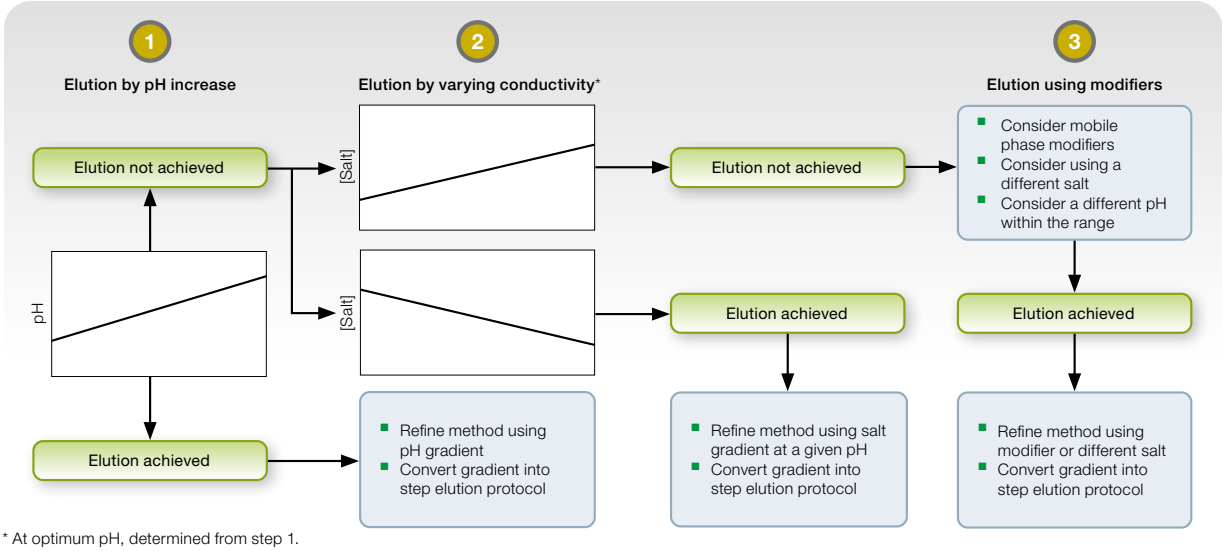
Fig. 3. Large design space afforded by Nuvia cPrime media.

Simple Method Development

The mixed-mode nature of the Nuvia cPrime ligand and its associated range of interactions allows for a directed and intuitive approach to method development and process optimization. Alternatively, a simple design of experiment (DOE) exercise will quickly guide developers to optimum loading, wash, and elution conditions afforded by the media's large design space (Figures 3 and 4).

Built to Meet the Demands of Commercial Operations

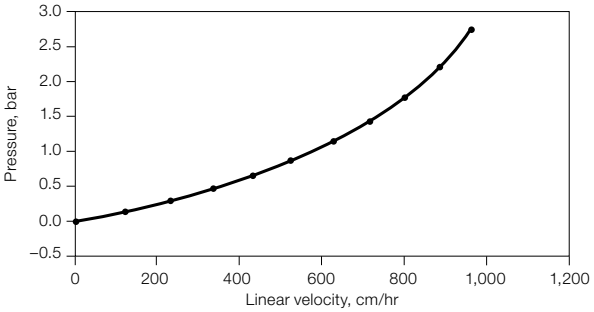
Nuvia cPrime is built on a porous polymeric base matrix that delivers low backpressure at high flow rates (Figure 5). It is also chemically and mechanically stable. Fast mass transfer dynamics ensure efficient chromatography at high flow, making Nuvia cPrime media an operationally superior choice for commercial-scale applications.



* At optimum pH, determined from step 1.

Fig. 4. Recommended approach to method development.

A. Column back pressure vs. linear velocity



B. Dynamic binding capacity vs. linear velocity, lactoferrin

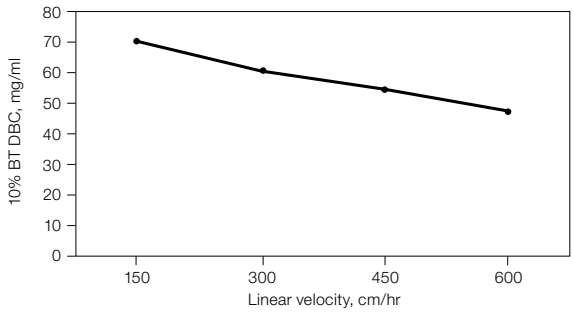


Fig. 5. Nuvia cPrime displays low backpressure at high flow rates. A, flow performance of Nuvia cPrime media in a Bio-Rad InPlace column. A 20 x 20 cm column with 17% axial compression was used. **B**, dynamic binding capacity vs. linear velocity of Nuvia cPrime media. A 1.1 x 9.6 cm column was loaded with 5.25 mg/ml lactoferrin in 20 mM NaOAc + 150 mM NaCl, pH 4.5, until 10% breakthrough was observed. BT, breakthrough; DBC, dynamic binding capacity.

Featured Products

Chromatography Resins and Screening Tools

Bio-Rad has a wide range of resins for each phase of virus process purification, at scales ranging from nanograms to kilograms. For more information, see the Virus purification resin selection card to help guide you to the best resin type for your process.

Request Sample

NGC Quest 10 Chromatography System

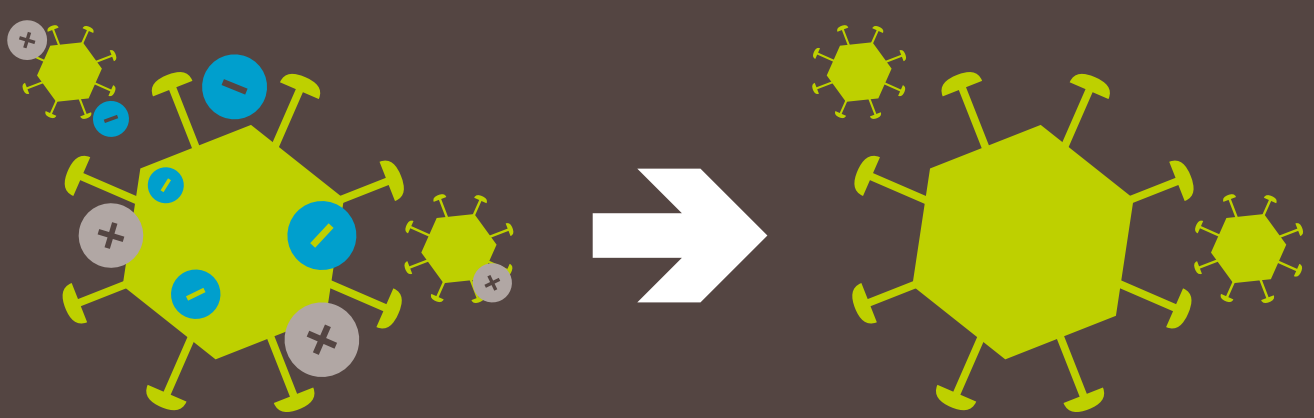
Ease of use: ★★★★★ **After sales service:** ★★★☆☆
Value for money: ★★★★☆

Rating: 4.0 ★★★★☆

Application Area:
Pesticides analysis with GC system

"A versatile piece of equipment that we can adapt for many different detectors"

Jose Luis Blasco Guillian, Boslan



Virus Purification Resin Selection Guide

Bio-Rad offers a wide range of resins for each phase of virus process purification, at scales ranging from nanograms to kilograms.

Use this guide to select the optimal resin for your virus process purification.

BIO-RAD

Visit **bio-rad.com/VirusChromResins** for more information.

Optimal Resin for Virus Purification

Property	Process Purification Resin Type					
	Adenovirus			Other Small to Midsized Mammalian Viruses		
Resin type	Nuvia cPrime	Nuvia Q	UNOsphere Q	CHT Ceramic Hydroxyapatite	CFT Ceramic Fluoroapatite	MPC Ceramic Hydroxyfluoroapatite
Chromatography type	Mixed-mode (HIC and cation exchange)	Anion exchange	Anion exchange	Mixed-mode (cation exchange and metal affinity)	Mixed-mode (cation exchange and metal affinity)	Mixed-mode (cation exchange and metal affinity)
Particle size	70 ± 10 µm	85 ± 15 µm	120 µm	20 ± 2, 40 ± 4, 80 ± 8 µm	40 ± 4 µm	40 ± 4 µm
Ionic capacity	110–150 µeq/ml	100–170 µeq/ml	120 µeq/ml	—	—	—
Dynamic binding capacity (DBC)	>40 mg hIgG/ml (at 10% breakthrough) at 300 cm/hr* >60 mg lactoferrin/ml*	≥170 mg/ml at 300 cm/hr*	≥180 mg BSA/ml at 150 cm/hr* ≥125 mg BSA/ml at 600 cm/hr*	≥25 mg lysozyme/g* 25–60 mg IgG/ml at 300 cm/hr*	14–21 mg lysozyme/g* 33 mg IgG/ml*	≥25 mg lysozyme/g* 25–50 mg IgG/ml*
Recommended linear flow rate	50–600 cm/hr	50–600 cm/hr	50–300 cm/hr	50–300 cm/hr	50–300 cm/hr	50–300 cm/hr
pH stability	Short-term: 3–14 Long-term: 4–13	Short-term: 2–14 Long-term: 4–12	1–14	6.5–14	Operating pH: 5–14 Storage pH: 11–14	6.5–14

BSA, bovine serum albumin; HIC, hydrophobic interaction chromatography.
Refer to bulletins 6790 and 6807 to see how these resins help in virus process purification.
* Go to the product detail page or bulletin 6713 on **bio-rad.com** to see how the DBC was determined and for other technical details.

Ordering Information

Nuvia cPrime	Catalog #	Size
Foresight Nuvia cPrime Plates	732-4705	2 x 96-well, 20 µl
Foresight Nuvia cPrime RoboColumn Unit	732-4807	200 µl
	732-4808	600 µl
Foresight Nuvia cPrime Column	732-4722	1 ml
	732-4742	5 ml
Nuvia cPrime Resin	1563401	25 ml
	1563402	100 ml
	156-3403	500 ml
	156-3404	1 L
	156-3405	5 L
	156-3406	10 L

Nuvia Q	Catalog #	Size
Foresight Nuvia Q Plates	732-4703	2 x 96-well, 20 µl
Foresight Nuvia Q RoboColumn Unit	732-4804	200 µl
	732-4805	600 µl
Foresight Nuvia Q Column	732-4721	1 x 1 ml
	732-4741	1 x 5 ml
Nuvia Q Resin	1560411	25 ml
	1560413	100 ml
	156-0415	500 ml
	156-0417	10 L

UNOsphere Q	Catalog #	Size
Foresight UNOsphere Q Plates	732-4714	2 x 96-well, 20 µl
Foresight UNOsphere Q RoboColumn Unit	732-4819	200 µl
	732-4820	600 µl
Foresight UNOsphere Q Column	732-4732	1 ml
	732-4752	5 ml
UNOsphere Q Resin	1560101	25 ml
	1560103	100 ml
	156-0105	500 ml
	156-0107	10 L

CHT Ceramic Hydroxyapatite	Catalog #	Size
Foresight CHT Type I Plates	732-4716	40 µm, 2 x 96-well, 20 µl
Foresight CHT Type II Plates	732-4718	40 µm, 2 x 96-well, 20 µl
Foresight CHT Type I RoboColumn Unit	732-4822	40 µm, 200 µl
	732-4823	40 µm, 600 µl
Foresight CHT Type II RoboColumn Unit	732-4825	40 µm, 200 µl
	732-4826	40 µm, 600 µl
Foresight CHT Type I Column	732-4735	40 µm, 1 ml
	732-4755	40 µm, 5 ml
Foresight CHT Type II Column	732-4736	40 µm, 1 ml
	732-4756	40 µm, 5 ml
CHT Type I Media, 40 µm	1584000	10 g
	1570040	100 g
	157-0041	1 kg
	157-0045	5 kg
CHT Type I Media, 80 µm	1588000	10 g
	1570080	100 g
	157-0081	1 kg
	157-0085	5 kg
CHT Type II Media, 40 µm	1584200	10 g
	1574000	100 g
	157-4100	1 kg
	157-4500	5 kg
CHT Type II Media, 80 µm	1588200	10 g
	1578000	100 g
	157-8100	1 kg
	157-8500	5 kg

CFT Ceramic Fluoroapatite	Catalog #	Size
Bio-Scale Mini CFT Type II Cartridges	7324405	1 x 5 ml
	7324406	5 x 5 ml
CFT Ceramic Fluoroapatite Type II Media, 40 µm	1585200	10 g
	1575000	100 g
	157-5100	1 kg
	157-5500	5 kg

MPC Ceramic Hydroxyfluoroapatite	Catalog #	Size
Foresight MPC Type I Plates	732-4785	40 µm, 2 x 96-well, 20 µl
Foresight MPC Type I RoboColumn Unit	732-4828	40 µm, 200 µl
	732-4829	40 µm, 600 µl
Foresight MPC Type I Column	732-4737	40 µm, 1 ml
	732-4757	40 µm, 5 ml
MPC Ceramic Hydroxyfluoroapatite Type I Media, 40 µm	1580200	10 g
	1570200	100 g
	157-0201	1 kg
	157-0205	5 kg

Ordering Information

Catalog #	Description
12006693	**Nuvia HP-Q Media**, 25 ml
12006691	**Nuvia HP-Q Media**, 100 ml
12006660	**Nuvia HP-Q Media**, 500 ml
12006659	**Nuvia HP-Q Media**, 5 L
12007023	**Nuvia HP-Q Media**, 10 L
12007020	**Foresight Nuvia HP-Q Column**, 1 ml
12007021	**Foresight Nuvia HP-Q Column**, 5 ml
12007013	**Foresight Nuvia HP-Q RoboColumn Unit**, 200 µl
12007014	**Foresight Nuvia HP-Q RoboColumn Unit**, 600 µl
12006908	**Foresight Nuvia HP-Q Plates**, 20 µl

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