Practical Guide: Selecting the Optimal Resins for Mammalian Virus Process Purification and Clearance

Payal Khandelwal, PhD
Bio-Rad Laboratories, Inc., 6000 James Watson Drive, Hercules, CA 94547

Purification Solutions

Bulletin 6790

Your One-Stop Shop for Mammalian Virus Purification Resins

Over the past 35 years or so, biologics such as monoclonal antibodies (mAbs), recombinant proteins, and, more recently, biosimilars and biobetters have become powerful therapeutics and been shown to be effective in treating various human diseases. However, as all biological products run the inherent risk of possibly carrying and/or amplifying extrinsic viral material, their successful implementation for use in humans depends on the ability to effectively remove any viral contaminants. Viruses can directly infect mammalian cells and cause diseases such as AIDS, mumps, measles, herpes, hepatitis, meningitis, and shingles. Therefore, viral clearance from biologics is critical to their performance. On the other hand, the purification and study of these disease-causing viruses can aid in the development of preventive and curative therapeutics. One of the most common process steps included in downstream virus purification and clearance schemes is the use of chromatography resins. Resins, unlike other conventional techniques, retain viral infectivity along with the quality and quantity of the virus purified and hence are the purification agents of choice.

Bio-Rad has provided a progressive selection of chromatography resins for the process-scale purification of viruses for over 50 years. Here, we provide a brief snapshot of the different apatite-based media that can be effectively utilized for the process-scale purification of small to mid-sized viruses. Their use is simple, easily scalable, and results in a concentrated preparation of highly active virus. For purification resins appropriate for large and highly complex viruses such as adenoviruses, refer to bulletin 6807 – Selecting the Optimal Resins for Adenovirus Process Purification.

CHT™ Ceramic Hydroxyapatite Media Can Be Used with Diverse Virus Types

CHT Ceramic Hydroxyapatite can be used for the chromatographic separation of enveloped and nonenveloped viruses of diverse sizes from different sources and different families, such as dengue, Japanese encephalitis, influenza, mouse hepatitis, adenovirus, poliovirus, and feline calicivirus (Table 1).

Table 1. Wide variety of viruses that can be purified using CHT.

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	ssRNA	–	90
Poliovirus	Picornaviridae	Enterovirus	ssRNA	–	30
Feline calicivirus	Caliciviridae	Vesivirus	ssRNA	–	30–38

Standard Virus Purification Protocol with CHT

The general workflow of virus process purification with CHT involves six steps as shown below with specific buffer requirements at each step (Table 2). 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 Media. Crude extract was run on the columns.

Wash → Equilibration → Sample loading → Wash → Elution → Wash

Table 2. Standard virus process 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

Viral Activity Is Retained with CHT Chromatographic Purification

Viruses purified with CHT were tested using multiple methods (Table 3) for retention of viral activity. The results show that CHT is able to purify highly active virus. Purity analysis for protein contaminants was carried out by UV absorbance at 280 nm and SDS-PAGE analysis and host cell DNA contamination was monitored using the Quant-iT PicoGreen dsDNA Assay Kit.

Table 3. Detection methods used to check viral activity after CHT chromatography.

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

Choosing the Right Apatite-Based Media

Bio-Rad offers two kinds of CHT Media, Type I and Type II, in multiple sizes. Type I has higher protein binding capacity and better capacity for acidic proteins while Type II has a lower protein binding capacity but offers better resolution of nucleic acids and certain proteins. We also offer MPC™ Ceramic Hydroxyfluoroapatite and CFT™ Ceramic Fluoroapatite, which have slightly different properties relative to CHT. Each of these resins behaves differently with different viruses under different conditions. Hence the choice of resin and the conditions used have to be optimized for the virus being purified.

For example, dengue virus type 2 was purified using all four apatites. The yield with MPC was only 50% versus ~80% yields from the other three apatites. Additionally, the best separation of virus from impurities was seen with CHT Type II Media due to its larger pore size (Figure 1). The importance of choosing the appropriate media for a specific virus was also seen when CHT Type II and CFT Type II Media were used in the purification of poliovirus. CHT gave a recovery of 88% while CFT recovered 102% (Figure 2) of the virus. For details about these purifications, refer to bulletin 6549.

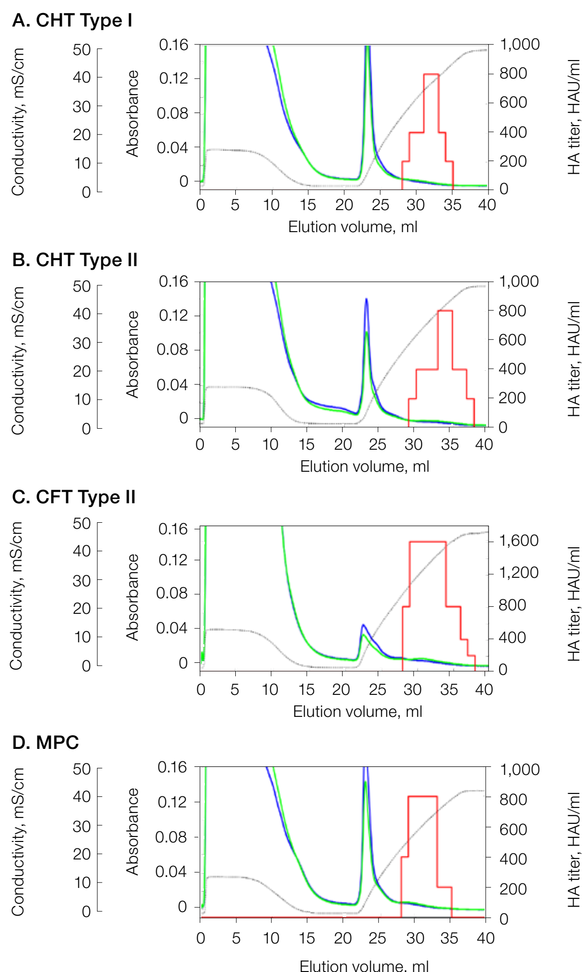

Fig. 1. Comparison of four apatites in the purification of dengue virus type 2. UV absorbance at 260 nm (—); absorbance at 280 nm (—); conductivity (--); viral activity in HA test (—).

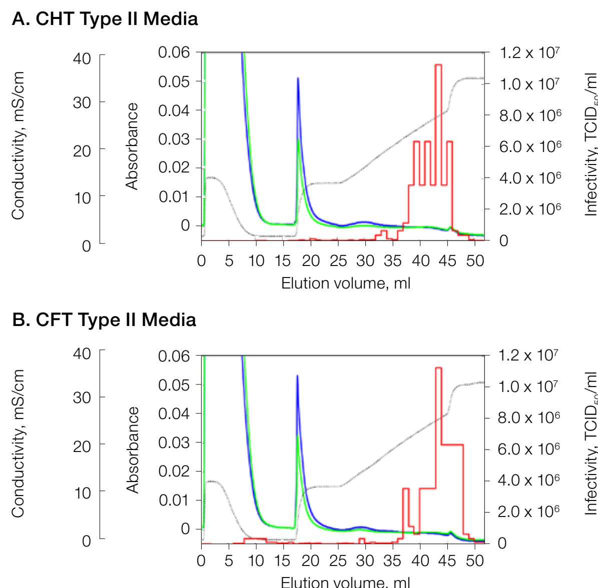

Fig. 2. Comparison of two apatites in the purification of poliovirus. UV absorbance at 260 nm (—); absorbance at 280 nm (—); conductivity (--); infectious activity in $TCID_{50}$ (—).

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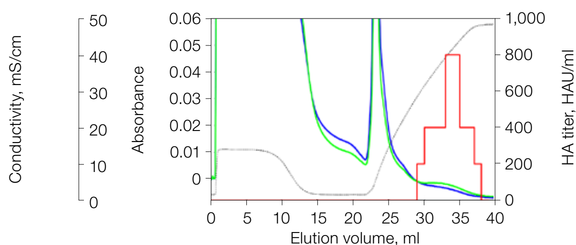
Selecting the Best Conditions for Virus Purification with Apatite-Based Media

Efficiency in viral purification in terms of time and quantity depends on multiple factors including the flow rate and the salt gradient slope. We show that decreasing the flow rate by tenfold (1.0 ml/min to 0.1 ml/min) improves the sharpness of elution peaks and hence the separation of dengue virus type 2 (Figure 3). For more details on the purification of the influenza, mouse hepatitis, and Japanese encephalitis viruses in a similar manner on various apatite media, refer to bulletin 6549.

A. 1.0 ml/min flow rate



B. 0.1 ml/min flow rate

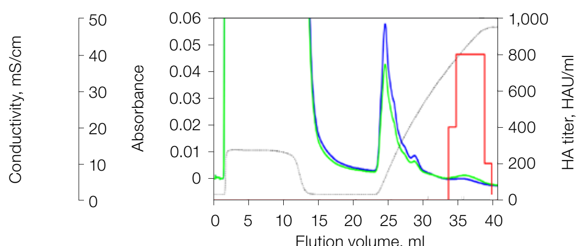


Fig. 3. Separation of dengue virus type 2 at various flow rates. UV absorbance at 260 nm (—); absorbance at 280 nm (—); conductivity (--); viral activity in HA test (—).

Hydroxyapatite media also support viral clearance of 0.6–3.5 logs (Dove et al. 1990, Grun et al. 1992). At times, they have also been shown to outperform size exclusion chromatography by a factor of 15, ion exchange chromatography by a factor of 75, and precipitation by a factor >150 in viral clearance (Dove et al. 1990). CHT, specifically, has been shown to clear up to 4 logs of xenotropic murine leukemia virus (x-MuLV) and 1–2 logs of minute virus of mice (MVM) from an antibody sample (Snyder et al. 2009). Therefore, they can be trusted as effective media for the purification and clearance of small to mid-sized mammalian viruses. As mentioned earlier, adenoviruses warrant the use of other mixed-mode and ion exchange resins such as Nuvia™ cPrime™, Nuvia™ S (cation exchange, CEX), Nuvia™ Q (anion exchange, AEX), UNOsphere™ S (CEX), and UNOsphere™ Q (AEX). The use of these resins for adenovirus purification is discussed in depth in bulletin 6807.

The details provided here can help you design a process purification strategy for your virus of choice. 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).

References

Dove GB et al. (1990). Purification alternatives for IgM (human) monoclonal antibodies. In ACS Symposium Series 427, M.R. Ladisch et al., eds. (Washington, DC: American Chemical Society), pp. 194–209.

Grun JB et al. (1992). Viral removal/inactivation by purification of biopharmaceuticals. Biopharm 5, 22–30.

Snyder MA et al. (2009). PEG enhances viral clearance on ceramic hydroxyapatite. J Sep Sci 32, 4048–4051.

Explore our extensive selection of process-scale chromatography resins and their performance characteristics and applications (bulletin 6713). For process optimization of your virus purification, request a sample.

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