

**NatriFlo® HD-Q Membrane Adsorbers:**

# Robust, load independent viral clearance in monoclonal antibody purification

**SUMMARY**

The single-use NatriFlo® HD-Q anion exchange membrane adsorber provides a simple, cost-effective and robust solution for final polishing and viral clearance of monoclonal antibodies, with far greater productivity than modern resins.

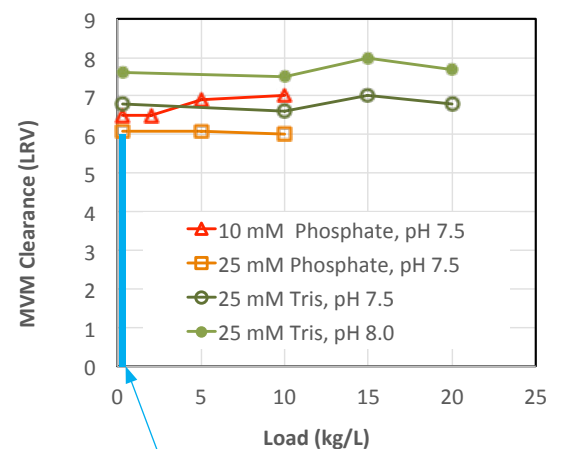
Anion exchange chromatography is an important flow-through polishing step for viral clearance of monoclonal antibodies. Resin based materials are most commonly used for this but the large size of virus particles limits diffusion and binding capacity. It is therefore necessary to use large volumes of resin and oversized column hardware. Column chromatography as a unit operation also means high operational costs, including validation, cleaning and storage. This is a particular issue for clinical manufacturing, where a column is used for only a fraction of its useful life.

The high charge density and open structure of the NatriFlo HD-Q material gives an exceptional dynamic binding capacity, even for virus particles. One NatriFlo HD-Q device can replace a chromatography column that is up to 80 times larger. For example, the 460 ml NatriFlo P600 HD-Q device can replace chromatography columns up to 40 liters.

**ROBUST, LOAD INDEPENDENT VIRAL CLEARANCE IN BOTH TRIS AND PHOSPHATE BUFFER**

The NatriFlo HD-Q shows load independent Minute Virus of Mice (MVM) clearance in both Tris and phosphate buffer (7.5/8 and 6 LRV respectively) with monoclonal antibody loads ranging from 0.25 to 20 kg/L of membrane and a 6 second residence time. The typical load for a modern resin is 0.1-0.4 kg/L of resin with residence times of 3-5 minutes.

Tris is the preferred buffer for anion exchanged based polishing of mAbs. Phosphate buffers are normally not recommended for this because the buffer ions bind to the resin and severely reduce its binding capacity. As shown here the HD-Q hydrogel is compatible with phosphate with only a modest reduction in clearance.

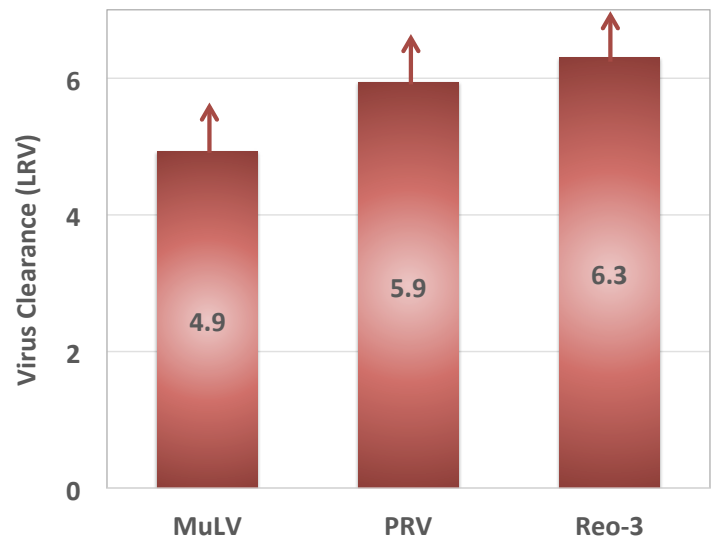


Note: The blue rectangle represents the typical operating limits of modern resin (0.3 kg/L load and 6 LRV)

**Figure 1:** Load independent MVM clearance in Tris and Phosphate buffer.

The sample, a partially purified biosimilar antibody (pI 8.4), had a concentration of 10 g/L with the conductivity adjusted to 10 mS/cm in all experiments.

Clearance of three other model viruses was also studied. The NatriFlo HD-Q material achieved LRV values of  $\geq 4.9$ ,  $\geq 5.9$  and  $\geq 6.3$  for Murine Leukemia Virus (MuLV), Pseudorabies Virus (PRV), and Reovirus type 3 virus (Reo-3) respectively. No breakthrough was observed for any virus using the standard sample load of 10 kg antibody/L membrane, 20 mM phosphate pH 7.5 buffer, 10 mS/cm, at 10 MV/minute. This further demonstrates the broadly applicable viral clearance properties of the material.

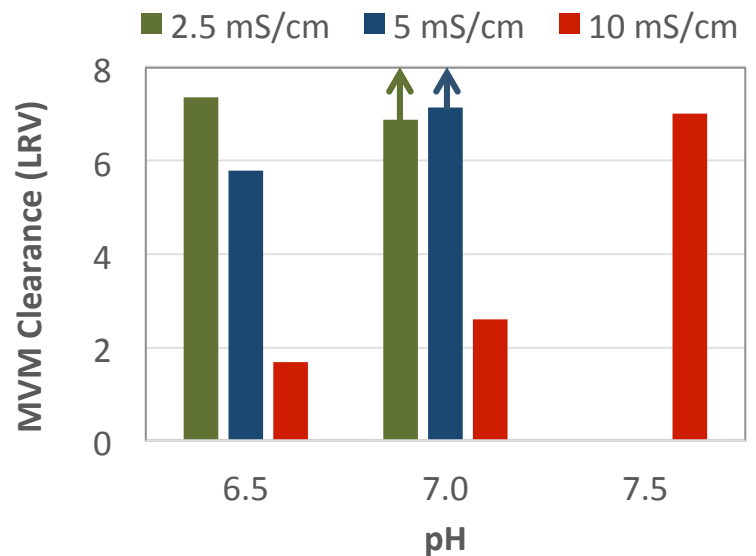


**Figure 3:** Reliable clearance of several different viruses.

The up arrows indicate limit of detection.

## EFFECT OF PH AND CONDUCTIVITY ON MVM CLEARANCE

With lower pH, as the buffer approaches the pI of the virus, the clearance goes down. This can be compensated for by lowering the buffer conductivity. 7 LRV MVM clearance was observed (10 mM phosphate buffer, pH 7.5, 10 mS/cm) using a sample load of 10 kg/L membrane and a flow rate of 10 MV/minute (6 seconds residence time). Similar clearance was also seen at pH 6.5 and 7 when the conductivity was lowered.

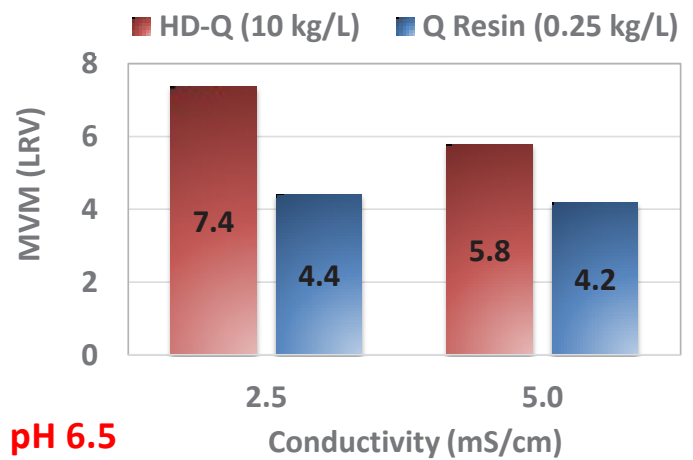


**Figure 4:** Robust MVM Clearance in varying buffer pH and conductivity.

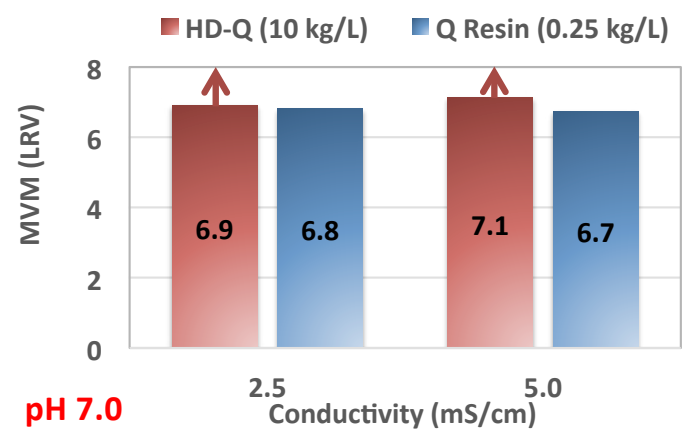
The up arrows indicate limit of detection.

## NATRIFLO HD-Q VS. MODERN Q-RESIN

The NatriFlo HD-Q device was compared to a modern Q-resin in phosphate buffer using MVM. It outperformed the Q-resin under all conditions tested. The difference was particularly pronounced at lower pH, where the NatriFlo HD-Q achieved up to 3 LRV greater clearance.



pH 6.5



pH 7.0

**Figure 4:** Comparison of MVM clearance by NatriFlo HD-Q and a modern Q-resin.

The up arrows indicate limit of detection.

## CONCLUSIONS

The NatriFlo HD-Q membrane adsorber is a cost-effective and robust single-use solution for antibody polishing. The superior viral clearance capabilities of the NatriFlo HD-Q membrane adsorbers were demonstrated by studying the clearance of the most difficult virus, MVM, over a wide design space. HD-Q also showed effective removal of three other viruses: xMuLV, PRV and Reo-3.

## MATERIALS AND METHODS

A biosimilar antibody with a pI of 8.4 expressed in Chinese hamster ovary (CHO) cells was used. It was captured from clarified cell culture supernatant using Ampsphere JWT Protein A resin (JSR Life Sciences). After a low pH virus inactivation step, the sample was processed using Poros XS cation exchange resin (Thermo Fisher Scientific) and then diafiltered into appropriate buffer. The virus spike ratio for all the experiments was 1% (v/v). NatriFlo HD-Q Recon Mini devices with an effective membrane volume of 0.2 mL was used as scale-down model for the entire virus clearance study. The column chromatography was done using a pre-packed anion-exchange column using high flow agarose resin with 10 cm bed height. All column work was done using 3 minutes of residence time as recommended by the supplier and a load of 0.25 kg mAb/L resin.

## REFERENCE

Data from a presentation by Horst Ruppach, Global Manager, Viral Clearance and Global Coordinator Virology, Charles River Labs, Bioprocessing Summit, August 2015

For additional information and performance data, please visit [www.natrixseparations.com](http://www.natrixseparations.com)

Natrix Separations, Inc.  
5295 John Lucas Drive | Unit 6  
Burlington, Ontario  
L7L 6A8 CANADA

TECHNICAL INQUIRIES:  
Contact Natrix Separations  
+1 905-319-2682  
[info@natrixseparations.com](mailto:info@natrixseparations.com)