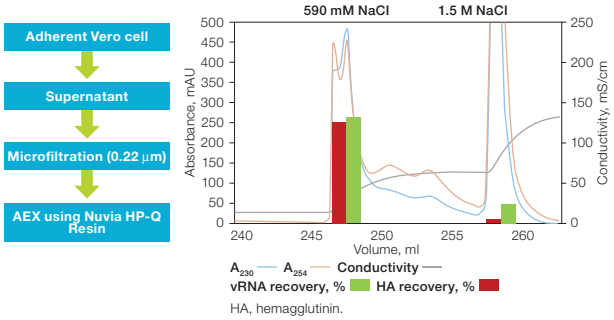
Quick Purification Strategy
H1N1 Purification

Efficient virus recovery and impurity removal are challenges in the purification of viruses such as influenza A virus subtype H1N1. As a capture chromatography medium, Nuvia HP-Q Resin resulted in high virus recovery and excellent impurity removal in a single purification step. Moreover, Nuvia HP-Q Resin does not require benzonase treatment or the intensive filtration step of H1N1 culture harvest before loading. Here, we summarize H1N1 purification on Nuvia HP-Q Resin.

Procedure

H1N1 Purification on Nuvia HP-Q Resin

Format: Foresight Nuvia HP-Q 1 ml Column, 5.83 x 10^{11} viral RNA (vRNA) copies/ml resin



HA, hemagglutinin.

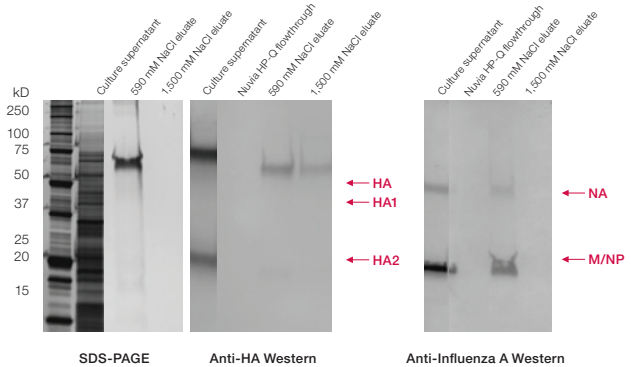
Optimized Protocol for Nuvia HP-Q Resin

Step	Mobile Phase	pH	CV	Remark
Equilibration	20 mM Tris, 100 mM NaCl	7.5	5	
Sample loading	20 mM Tris, 100 mM NaCl	7.5	–	5.83 x 10^{11} viral RNA copies/ml of resin
Wash	20 mM Tris, 100 mM NaCl	7.5	10–30	
Elution	20 mM Tris, 590 mM NaCl	7.5	10	
High salt strip	20 mM Tris, 1.5 M NaCl	7.5	5	
Clean in place	1 M NaOH	>12	10	

* Operational linear flow rate: 84 cm/hr.

Results

SDS-PAGE and Western Blot Analyses



Average size: 128 ± 4.18 nm
Reported size range: 80–180 nm

Purified H1N1 virus was highly monodispersed with 99.8% monomer with a diameter of 128 nm.

Data generated by Bioprocessing Technology Institute (BTI) Singapore.

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Results

Purification Step	HA Recovery	HCP/HA, mg/mg	Host cell DNA/HA, ng/mg
Culture supernatant	100.0	13.9	442.5
Microfiltration	100.0	13.0	221.3
Nuvia HP-Q eluate	80.0	1.1	0.3
EP guideline (for 15 HA dose)*	–	≤6.7	≤0.67

* ema.europa.eu/en/documents/scientific-guideline/guideline-influenza-vaccines-quality-module-revision-1_en.pdf
HA, hemagglutinin; HCP, host cell protein.

Highlights

- Benzonase treatment not required in this workflow
- High binding capacity
- Effective impurity removal

Reported Purification Schemes	HA Recovery, %	HCP/HA, mg/mg	hcDNA/HA, ng/mg	Reference, DOI
Diafiltration/microfiltration/ultrafiltration size exclusion chromatography, anion exchange chromatography (AEC)	53.0	7.7	34.5	10.1002/bit.21109
AEC, hydrophobic interaction chromatography	92.2	17.2	2.0	10.1016/j.jchromb.2019.03.037
AEC monolith	89.0	NA	NA	10.1016/j.jchromb.2017.06.086
Pseudo-affinity (saturated membrane)	94.2	2.1	23.0	10.1002/bit.22345
Pseudo-affinity (saturated membrane)	64.0	13	3.8	10.1002/jctb.5474

Visit **bio-rad.com/NuviaHP-Q** to get technical details about the resin used in this workflow.

Visit **bio-rad.com/ProcessApplications** to see other applications in which these resins can be used.

Visit **bio-rad.com/processlibrary** to speak with a scientist or request a customized purification seminar.

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Bulletin 7375 Ver A US/EG *20-0450 0820 Sig 0220*