Ready to implement CIM® Monolith Technology

Order our CIM® Disk Virus Purification Pack and identify the optimal chemistry for your virus purification. You can order our Virus Purification Pack online at www.monoliths.com/catalog or by contracting your local BIA Separations Sales representative.

Request a CIM® Technology Seminar?

To educate your entire organization about CIM® Technology and its capabilities in biomolecule purification request a CIMinar™. Just write us at sales@monoliths.com to begin solving your purification challenges.

For any additional information please contact us:

EUROPE

PRODUCTION:
BIA Separations d. o. o.
Teslova 30, SI-1000 Ljubljana, Slovenia, EU
Tel.: +386 1 426 5649
Fax: +386 1 426 5650
sales@biaseparations.com
orders@monoliths.com
www.biaseparations.com

SALES:
BIA Separations GmbH
Europastrasse 8
9524 Villach, Austria
Tel.: +386 1 426 5649
Fax: +386 1 426 5650
sales@biaseparations.com
www.biaseparations.com

USA

SALES:
BIA Separations, Inc.
1000 N. West St. Suite 1200
Wilmington, DE 19801, USA
Phone: 1-888-520-4CIM (4246)
Fax: 1-302-861-0362
sales@biaseparations.com
www.biaseparations.com

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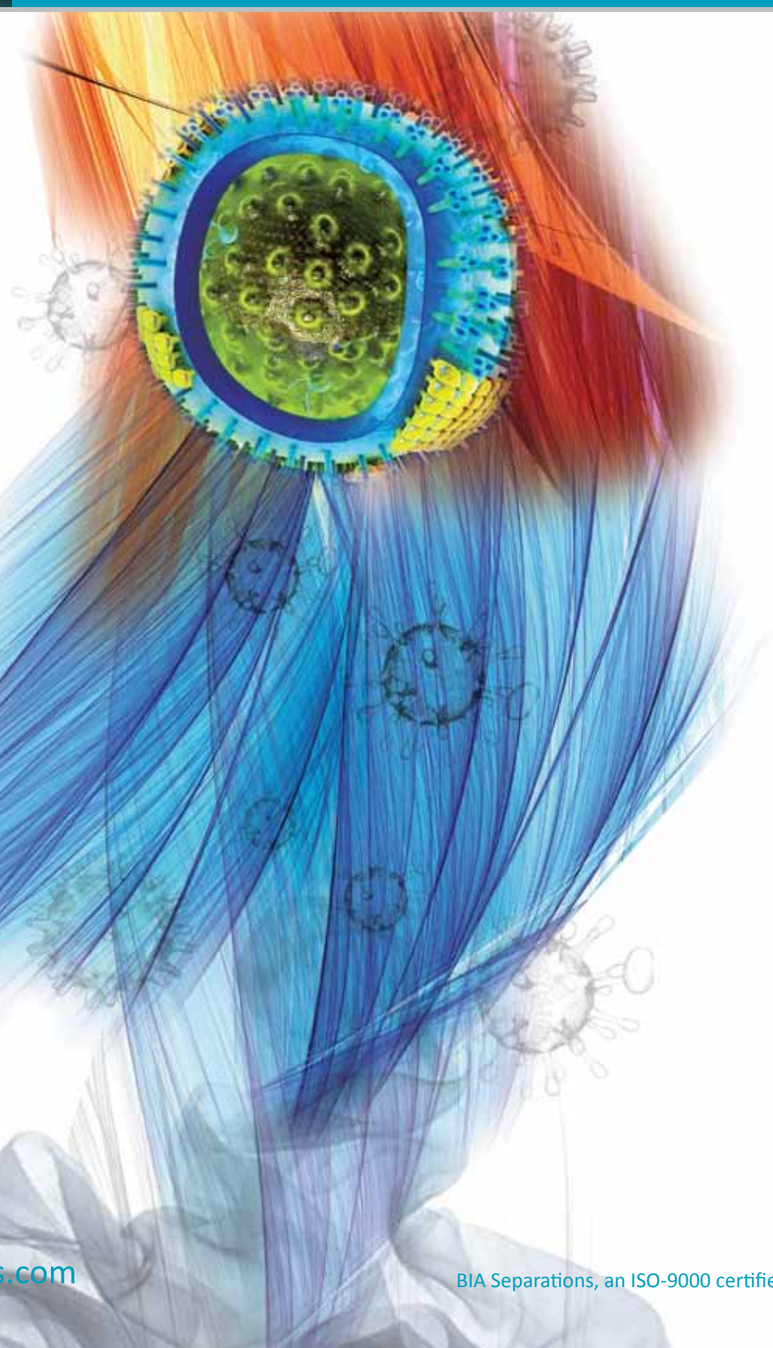
Leaders in Monolith Chromatography

CIM Convective Interaction Media®

VIRUS DOWNSTREAM PROCESSING USING CIM® MONOLITHS



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Leaders in Monolith Chromatography

AAV
Influenza A&B
ToMV
Rotavirus
HSV
Rabies
Bacteriophages
Hepatitis
Measles virus
Rotavirus

CIM® Monolithic Columns

SPECIALLY DESIGNED FOR VIRUS PURIFICATION.

Monoliths' convective interaction offer:
- extremely high binding capacity for viruses (up to 10^{14} VP/mL)
- accelerated process development
- increased manufacturing capacity
- preserved virus biological activity (low shear forces)

CIM$_{ac}$™ 0.1 mL
Recommended flow rates:
0.2 - 2 ml/min

CIM® 0.34 mL
Recommended flow rates:
1 - 8 ml/min

CIM® 1 mL
Recommended flow rates:
1 - 16 ml/min

CIM® 8 mL
Recommended flow rates:
10 - 200 ml/min

CIM® 80 mL
Recommended flow rates:
40 - 250 ml/min

CIM® 800 mL
Recommended flow rates:
400 - 2000 ml/min

CIM® 8000 mL
Recommended flow rates:
2000 - 10000 ml/min

Analytical Scale						
	Screening/Method Development					
			Preparative Scale			
				Large Scale Production		

Virus Purification & Vaccine Production Made CIMple™

Can you imagine a better way to respond to a Flu pandemic than purifying 10 million doses of vaccine in just 30 minutes? Can you imagine getting your vaccine to market 1-2 years earlier? Using CIM® Monoliths in your Downstream Process (DSP) can give you the competitive edge that you need.

CIM® Monoliths' high dynamic binding capacities and high flow rates can achieve levels of productivity in vaccine production unrivaled by traditional ultracentrifugation methods. CIM® Monoliths provide high returns by being more productive at a lower cost than using traditional methods and are better suited to meet the increasing economic and regulatory demands for improved vaccine production.

Live Influenza Vaccine Downstream Processing

Today, CIM® Monoliths are changing the paradigm for the production of Live Influenza Vaccine.

Using CIM® Monoliths in your DSP provides up to a tenfold increase in produced doses of flu vaccine without a large capital investment.

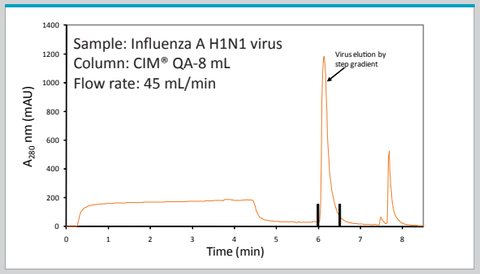
You could purify 10 million doses of vaccine in as little as 30 minutes.

CIM® QA Monolithic columns are an easy way to increase the number of doses on a given scale while maintaining excellent purity (99.96 % DNA removal, 97.8 % Protein removal) as compared to ultracentrifugation (see Table 1). And due to CIM® monolithic columns high binding capacity, viruses are eluted in smaller volume at higher concentration; further improving manufacturing productivity.

Table 1. Comparison of Purification Methods[1]

Standard UCF Method		CIM® Monolith Method	
Tangential Flow Filtration (TFF)		TFF	
↓		↓	
Ultracentrifugation (UCF)		(AIEX) CIM® QA Tube Monolithic Column	
↓		↓	
Adjustment to final formulation		Size Exclusion (SEC)	
Infectious virus yield	11.40%	Infectious virus yield	47.30%
DNA removal	99.50%	DNA removal	99.96%
Protein removal	97.40%	Protein removal	97.80%

Figure 1. Purification of Influenza H1N1 virus



[1] Maurer E., Peterka M., Gassner M., Seper H., Gelhart F., Banjac M., Jarc M., Lah B., Kramberger P., Štrancar A., Muster T. 2008. Influenza vaccine purification platform. Lecture presented at Monolith Summer School, Slovenia, Portoroz.

AAV1 Downstream Processing

This clinical grade AAV1 purification process produced 1x10^{15} purified AAV1 DNase Resistant Particles (DRP) in a few hours with 59% overall process recovery. The DSP was scaled to accommodate a 10 L production run in an extremely short time.

rHSV co-infection of cells
↓
Media exchange
↓
Lysis and primary clarification
↓
Concentrate and buffer exchange
↓
CIM® QA
↓
AVB Sepharose
↓
Concentrate and buffer exchange
↓
Filter, formulate & store

Table 2. Production and purification process overview[2]

Load (DRP/mL)	Elution (DRP/mL)	Recovery	Overall Process Recovery
1.8 x10^{11}	2.6 x10^{12}	73%	59%

Assay	Result
AAV concentration (DRP/mL)	6.8×10^{12}
AAV infectivity ($TCID_{50}$/mL)	2.0×10^{11}
Residual BHK DNA (ng/mL)	1.5
Purity (silver stain)	VP1, 2, 3 only
Residual HSV DNA (ng/mL)	190
Residual HSV protein (ng/mL)	60
Total protein (>µg/mL)	270
Endotoxin (EU/mL)	0.13
Bioburden	< LOD (5 cf/mL)
In vivo activity (ng protein/mL serum)	3.8×10^{4}
DRP into C57BL/6 mice, n=12	

Adenovirus Downstream Processing

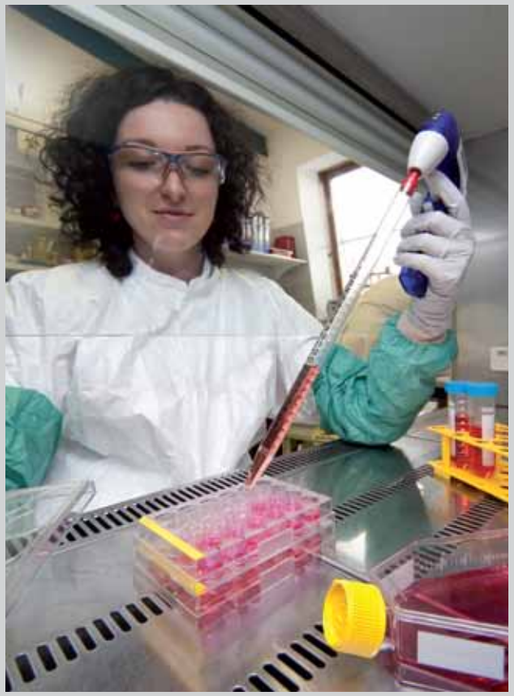
When comparing different purification methods for infective Adenovirus, CIM® Monoliths have threefold higher recoveries at twice the speed, than a membrane based purification kit (See Table 3). CIM® Monoliths also have better DNA removal.

Table 3. Purification of infective Adenovirus:
CIM® Monoliths Vs. Membrane based purification kit

	CIM® QA Disk Monolithic Column (chromatography based method)	Membrane based purification kit (centrifuge based method)
	Virus recovery (%)	**Virus recovery (%)**
EPD	**66.7**	21.1
NTA	**57.9**	4.1
	Removal (%)	**Removal (%)**
BU (proteins)	**99.4**	99.7
PG (DNA)	**91.6**	87.3
Total time	**66 min**	105 min

Sample: Ad harvest (freeze-thaw of the whole harvest followed by centrifugation)

EPD (End Point Dilution Assay) - infectious virus particles [pfu/ml]
NTA (Nanoparticle Tracking Analysis) - total virus particles [VP/ml]
BU (Bradford Ultra Assay) - total proteins
PG (PicoGreen Assay) - total DNA



[2] Wang L., Niamke J., Veres G., Knop D.R., Two-step chromatography purification of rAAV1 vectors by suspension BHK cells rHSV co-infection.

Bacteriophage Purification

By identifying the correct CIM® Monolithic Column (QA, DEAE or SO3), a high degree of bacteriophage purity with capacities reaching 2 x 10^{13} pfu/mL enabling purification of 1.6 x 10^{17} phages in one run on 8 L column are easily obtained. Multiple bacteriophage strains have already been purified with CIM® Monolithic Columns (See Figure 2). Successful purification has been achieved with bacteriophages that differ in: size (T7, M13), by host bacteria (*E.coli*, *S. aureus*…), by the charge of the protein structure (T7, T4…) or by type of life cycle (filamentous – M13, lytic – Lambda phage).

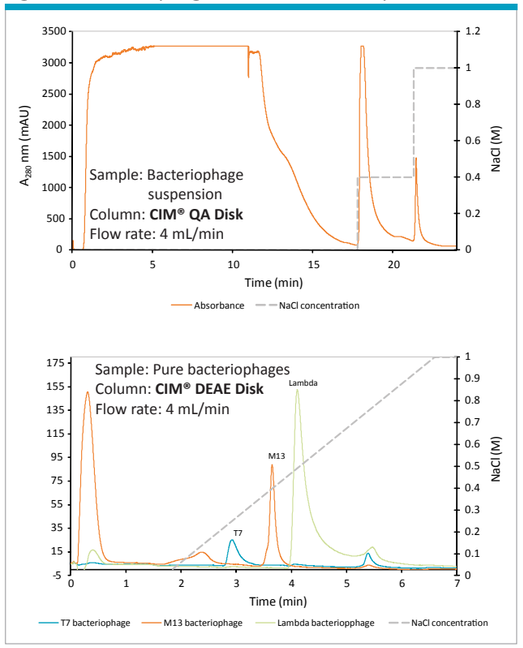
Bacteriophage recovery	65 %
Protein depletion	≥ 99 %
DNA depletion	≥ 90 %

* Purification of the Staphylococcus aureus phage[3]

Dynamic Binding Capacity (DBC)
2 x 10^{13} pfu/mL of monolithic resin

* Purification of bacteriophage M13[4]

Figure 2: Bacteriophage concentration and purification

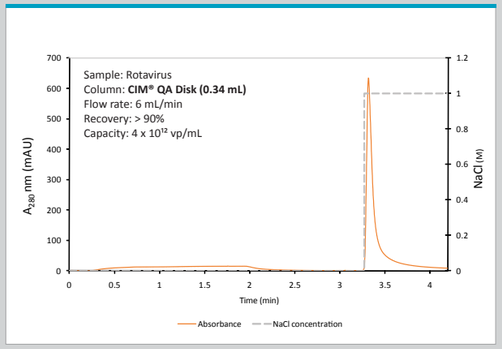


Other Viruses

Rotavirus, Rabies virus, Lentivirus, Measles, HSV, Mumps, CMV, MVA and many others can be successfully purified with CIM® Monolithic Columns.

Rotavirus

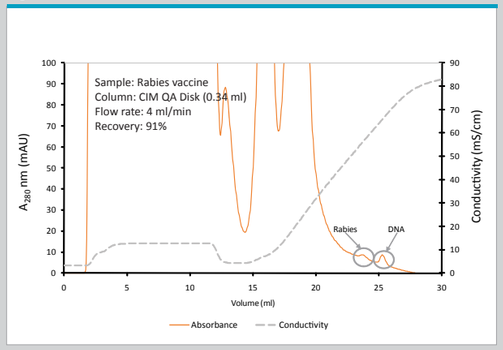
Figure 3: Rotavirus purification



CIM® QA Monolithic Columns can be used for work on Rotavirus as well. As an example, purification and concentration of Rotavirus is demonstrated in Figure 3[5].

Rabies Vaccine

Figure 4: Rabies vaccine purification



High resolution, high efficency, high flow rate separation of Rabies vaccine and DNA. Monoliths separate both despite similar elution conditions and low titers.

To learn how CIM® Monoliths can help you purify your target virus contact us at tech-support@monoliths.com.

3 Kramberger P., Honour R.C., Herman R.E., Smrekar F., Peterka M., 2010. Purification of the Staphylococcus aureus Bacteriophage 5 VDX-10 on Methacrylate Monoliths. Presented at the MSS2010, Portorož, Slovenia.
4 Smrekar F., Lunder M., Podgornik A., Štrancar A., 2009. Bacteriophage purification using CIM monolihts. Presented at the Phage in Interaction, Leuven, Belgium.
5 Gutiérrez-Aguirre I., Banjac M., Steyer A., Poljšak-Prijatelj M., Peterka M., Štrancar A., Ravnikar M. 2008. Concentrating rotaviruses from water samples using monolithic chromatographic supports. Presented at the MSS2008, Portorož, Slovenia.

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Purification Process Development

BIA Separations' Contract Research Laboratory has expertise in virus, pDNA, and monoclonal antibody downstream purification process development. Our team can deliver a robust and efficient purification process which will meet your company's and local regulatory agency's requirements.

Please feel free to contact us at sales@biaseparations.com should you wish to avail yourselves of our services. By taking advantage of CIM® monoliths we are able to rapidly develop processes in our Biosafety Level 2 laboratories.

Meeting Regulatory Demands

To meet the strictest regulatory demands of agencies worldwide, BIA Separations produces cGMP compliant Columns (stainless steel or disposable). It is very easy to move from method development to pilot and full scale cGMP production as CIM® monoliths have an identical performance profile regardless of scale. We currently have Drug Master Files for the chemistries (QA, DEAE, and SO3) that are used in Vaccine Production. Others Drug Master Files are being prepared.

BIA Separations, an ISO-9000 certified company.