A045 Purification of influenza A virus from embryonated chicken eggs using CIMac™ Adeno-0.1 Analytical Column

Influenza vaccines are still predominantly produced in embryonated chicken eggs and the purification processes barely have changed during the years. There is a growing need for fast, efficient and economical vaccine production.

So far, monolithic supports have been used successfully in virus purification and concentration, as well as in the purification of virus-like particles (VLP) propagated in cell cultures.

Therefore, our aim was to prove the applicability of monoliths in purification of influenza virus A propagated in embryonated chicken eggs.

SAMPLE PREPARATION

Influenza A virus (equine H3N8 strain) was inoculated into the allantoic cavity of 10 day old embryonated chicken eggs.

INOCULATION of Influenza A virus into embrionated eggs → **INCUBATION** at 37°C, 72 h → **VIRUS HARVEST** (collection of allantoic fluid) → **CLARIFICATION** (centrifugation; 2500 rpm for 15 min) → **STORAGE** at -70°C

RESULTS

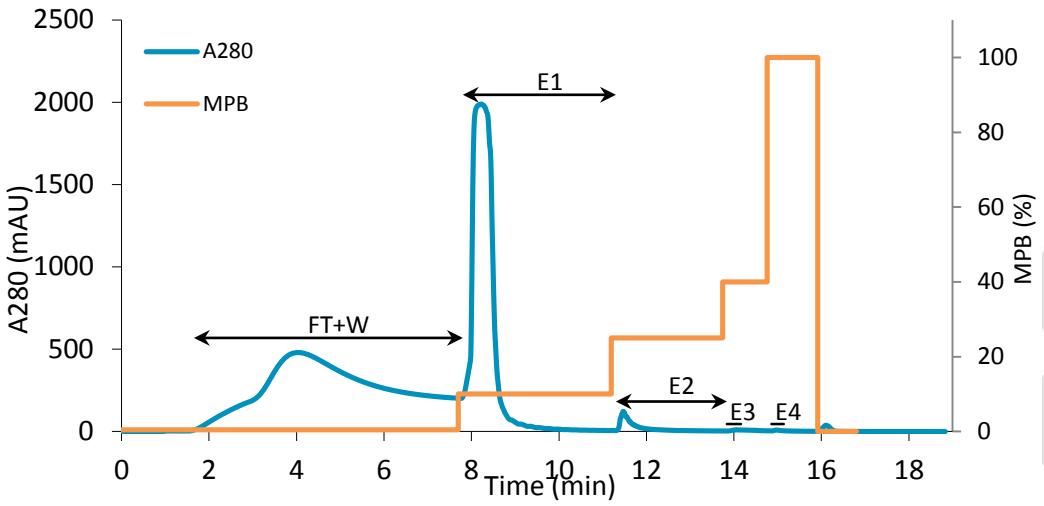
> Figure 1

Step gradient elution of influenza virus harvest

Column:	CIMac™ Adeno-0.1 Analytical Column (Pores 2 um); Catalog number: 110.8502-2
Column chemistry:	Strong anion exchanger; quaternary amine (QA)
Instrumentation:	HPLC Knauer system
Mobile phases:	Buffer A (MPA): 50 mM HEPES; pH 7.5 Buffer B (MPB): 50 mM HEPES containing 2 M NaCl; pH 7.5
Loading material preparation:	The virus harvest was diluted 10 times in equilibrating mobile phase (MPA) and filtered through 0.45 µm CA membrane filters.
Column loading volume:	1.32 mL
Flow rate:	1 mL/min
Method:	Step gradient elution: 0.2 M – 0.5 M – 0.8 M – 2M NaCl in MPA
UV detection:	UV at 280 nm
Virus quantification:	HA assay
Protein quantification:	Bradford Ultra assay
DNA quantification:	PicoGreen assay

> Figure 2

Step gradient elution chromatogram of influenza virus on CIMac™ Adeno-0.1 Analytical Column



> Table 1

Recovery of influenza virus, proteins and DNA during the purification process

Fraction	Virus's HA titer (% of load)	Protein (% of load)	DNA (% of load)
Load	100	100	100
FT + W	0	15	5
E1	0	68	23
E2	88	13	10
E3	6	0	42
E4	**0**	**0**	**4**
Total recovery	94	96	84

CONCLUSIONS:

An efficient purification process for influenza A virus obtained from allantoic fluid was developed on CIMac™ Adeno-0.1 Analytical Column (quaternary amine). High virus yield (88%) and efficient depletion of proteins (87 % total proteins) and DNA (90% total DNA) was achieved.

CIM® technology enables fast and efficient one step purification of influenza A virus produced in embryonated chicken eggs.

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