BIA separations

NIB

CIM Convective Interaction Media®

APPLICATION NOTE

A037

CIM® QA Monolithic Column for enrichment of virus particles from clinical samples prior to next-generation sequencing (NGS)

Orthoreoviruses are dsRNA, non-enveloped viruses that can cause severe enteric and respiratory infections in humans and other animals. It is speculated that these viruses might be an important zoonotic pathogen. As such, orthoreoviruses can cause infections of undetermined etiology which are difficult to resolve.
Next-generation sequencing (NGS) is a new technology which enables gathering a huge amount of genomic information from a sample in a short period of time. NGS is being increasingly applied in animal screenings for pathogen discovery and has a great potential in clinical microbiological diagnostics. However, the preparation of high-quality and high-quantity nucleic acid samples is a major concern for efficient application of the method.

CIM QA® disk in combination with NGS was used for discovering a novel reovirus in stool samples of a child with gastroenteritis infection of undetermined etiology. Two different starting samples were compared: clarified stool suspension and supernatant from cell culture inoculated with clarified stool suspension.

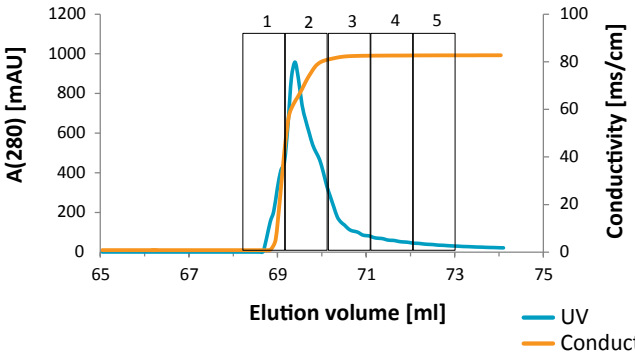
RESULTS:

> Figure 1

CIM® one-step elution of orthoreoviruses from a clarified stool suspension pretreated with benzonase

Loading material preparation: a 10 % stool suspension was prepared and clarified by centrifugation (1600 x g). Supernatant was filtered through a 0.22 µm pore filter (Millipore, Billerica, MA) and incubated with 1000 U/ml of Benzonase (Novagen, San Diego, CA) for 1h at room temperature. The sample was then diluted 6 times in a chromatography running buffer (Buffer A) and left overnight at 4 ˚C.

Column:	CIM® QA Disk Monolithic Column (Quaternary amine; CV: 0.34 ml)
Instrumentation:	AKTA purifier chromatographic system (GE Healthcare, Uppsala, Sweden)
Column loading volume:	10 ml
Mobile phases:	Buffer A: 50 mM HEPES; pH 7 Buffer B: 50 mM HEPES containing 1 M NaCl; pH 7
Elution:	One step gradient at 1 M NaCl (Buffer B)
UV detection:	UV at 280 nm
Elution fraction volumes:	1 ml
Virus particle measurment:	virus particle counting under an electron microscope using the negative staining technique
Cell host DNA:	18S RT-qPCR



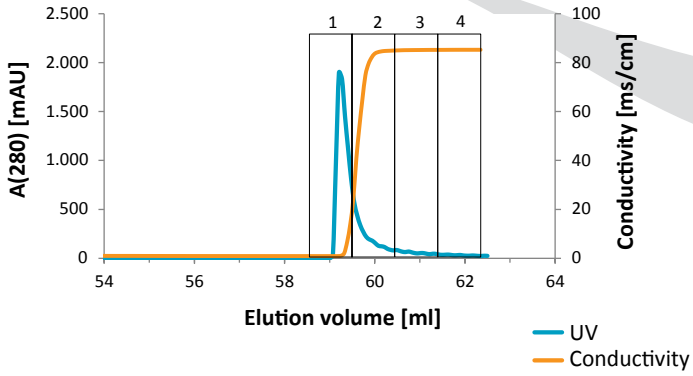
Fraction	Ct(18S RT -qPCR)	Virus/Field
Load	34,87	<1
1	35,45	<1
2	35,02	<1
3	34,63	<1
4	34,64	<1
5	Not tested	Not tested

> Figure 2

CIM® one-step elution of Orthoreoviruses from a cell culture supernatant pretreated with benzonase

Loading material preparation: the cell culture supernatant was incubated with 1000 U/ml of Benzonase (Novagen, San Diego, CA) for 1h at room temperature. The sample was then diluted 6 times in a chromatography running buffer (Buffer A) and left overnight at 4 ˚C.

Column:	CIM® QA Disk Monolithic Column (Quaternary amine; CV: 0.34 ml)
Instrumentation:	AKTA purifier chromatographic system (GE Healthcare, Uppsala, Sweden)
Column loading volume:	10 ml
Mobile phases:	Buffer A: 50 mM HEPES; pH 7 Buffer B: 50 mM HEPES containing 1 M NaCl; pH 7
Elution:	One step gradient at 1 M NaCl (Buffer B)
UV detection:	UV at 280 nm
Elution fraction volumes:	1 ml
Virus particle measurment:	virus particle counting under an electron microscope using the negative staining technique
Cell host DNA:	18S RT-qPCR



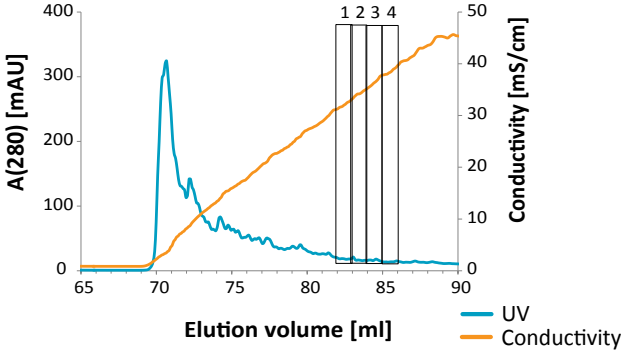
Fraction	Ct(18S RT -qPCR)	Virus/Field
Load	33,12	1
1	32,29	12
2	31,95	22
3	34,75	12
4	32,59	Not tested

> Figure 3

CIM® gradient elution purification of orthoreoviruses from a cell culture supernatant (without benzonase pretreatment)

Loading material preparation: the cell culture supernatant was diluted 6 times in a chromatography running buffer (Buffer A) and left overnight at 4 ˚C.

Column:	CIM® QA Disk Monolithic Column (Quaternary amine; CV: 0.34 ml)
Instrumentation:	AKTA purifier chromatographic system (GE Healthcare, Uppsala, Sweden)
Column loading volume:	10 ml
Mobile phases:	Buffer A: 50 mM HEPES; pH 7 Buffer B: 50 mM HEPES containing 1 M NaCl; pH 7
Elution:	Linear gradient from 0 to 500 mM NaCl in 60 CV
UV detection:	UV at 280 nm
Elution fraction volumes:	1 ml
Virus particle measurment:	virus particle counting under an electron microscope using thenegative staining technique
Cell host DNA:	18S RT-qPCR



Fraction	Ct(18S RT -qPCR)	Virus/Field
Load	25,10	4
1	35,08	33
2	32,64	59
3	33,78	50
4	31,57	15

Figure 4

Quality and quantity of nucleotide sequences obtained with Ion Torrent Sequencing (NGS)

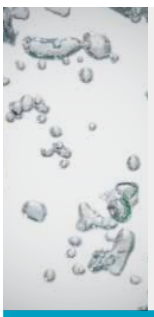
		Sample 1 (stool) benzonase CIM one - step elution	Sample 2 (cell culture) benzonase CIM one step elution	Sample 3 (cell culture) no benzonase CIM gradient elution
Number of reads		199.352	521.242	140.621
De novo assembly	N50	155	88	236
	N of contigs	885	2556	371
	N (and total length) of regions not covered by contigs	73 (4787 nt)	96 (6258 nt)	9 (89 nt)
Mapping to new Orthoreovirus consensus sequence	% of reads mapped	4.91	33.4	40.14
	Average coverage	11.47x (SD: 5.49x)	151.77x (SD: 83.42x)	119.07x (SD: 46.06x)
	Average coverage excluding duplicates	10.08x (SD: 4.35x)	39.34x (SD: 12.11x)	76.74x (SD: 21.17x)
	N (and total length) of zero coverage regions (all)	6 (17 nt)	4 (5 nt)	4 (5 nt)
Mapping to Macaca mulattta genome	% of reads mapped	0.03	30.83	2.54

Conclusions:

Using the CIM® QA Disk Monolithic Column with strong anion exchange, ligand reoviruses were efficiently concentrated from clinical samples and separated from host cell DNA impurities.

Purification with the CIM® QA Disk Monolithic Column in a linear gradient was the most effective: even though the initial material was not pretreated with benzonase, the host cell DNA was efficiently removed (increase in Ct values for 18S from 25 in the load to up to 35 in the virus-rich fractions leads to 3-orders-of-magnitude reduction of the background RNA), and the highest concentration of virus particles was obtained. The NGS results were also the best after a linear gradient in the CIM® QA disk, with highest number of the total reads mapping to the orthoreovirus consensus sequence. The method proved to be an efficient pretreatment step in NGS for virus diagnostics from clinical samples when the target viral pathogen is expected to be present within a high nucleic acid background.

More details can be found in the following article:
A. Steyer, I. Gutiérrez-Aguire, M. Kolenc, S. Koren, D. Kutnjak, M. Pokorn, M. Poljsak-Prijatelj, N. Racki, M. Ravnikar, M. Sagadin, A. Fratnik Steyer and N. Toplak. High Similarity of Novel Orthoreovirus Detected in a Child Hospitalized with Acute Gastroenteritis to Mammalian Orthoreoviruses Found in Bats in Europe. Journal of Clinical Microbiology, 2013, 51(11):3818.



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