



Proteomix[®] AAV SAX

***Improving Empty and Full AAV Capsids
Separation and Quantitation with UV-MALS***

— Sepax Technologies, Inc. —



Adeno-associated viruses (AAV) are one of the most commonly used viral vectors for gene therapy to deliver therapeutic genes. Quantitation of **empty and full AAV capsids** is critical to understanding and ensuring product quality, safety, and potential efficacy. Multiple techniques have been used in this application however all have some shortcomings and challenges:

- **Time-consuming:** Analytical ultra-centrifugation (AUC) is the classical and golden standard technique for this application; however, it requires long hours of processing time and a large amount of sample consumption.
- **Lack of accuracy:** UV absorbance at 260 nm versus 280 nm has also been widely used for the quick estimation of empty and full capsids, but the accuracy can be compromised. Traditional anion exchange chromatography (AEX) based on relative surface charge differences was also reported to provide the potential separation; however, the AEX analytical products on the market often lack accurate quantitative measurement to demonstrate the full separation of the empty and full capsid. Empty capsids may still be present in the AUC run of the fraction collected from the full capsid peak.



By improving its beads technology and surface coating chemistry, Sepax introduces a new **Proteomix® AAV SAX** MALS columns, which are specifically designed for **optimal separation of empty and full AAV capsids** delivering a **fast and accurate** quantitation method for analytical characterization and quality control.



Technical Specification



Phases	<i>Proteomix</i> [®] AAV SAX
Resin Matrix	Highly cross-linked monosized PS/DVB
Particle size	3 µm
Pore structure	Non-porous
Functional group	Quaternary ammonium
Dynamic binding capacity	~36 mg/mL
pH stability	2 – 9, 9.5*
Operating temperature limit	80 °C
Resin pressure limit	8000 psi
Column operating pressure limit	6,000 psi (a 4.6 x 50 mm PEEK column)
Typical flow rate	0.1-1.0 mL/min (for a 4.6 x 50 mm PEEK column)
Mobile phase compatibility	Compatible with aqueous solution, a mixture of water and acetonitrile, acetone, or methanol. Typical buffers: phosphate, tris, acetate, and so on.

*Extended use at higher pH levels (≥ 9.5) may reduce the column's lifetime.



A fast, easy and accurate quantitative analysis with excellent lot-to-lot consistency:

- **Improving Separation of Empty and Full AAV Capsids:** Achieved higher resolution separation of full capsids from empty capsids, allowing for full separation and more accurate molecular weight calculations in the applications.
- **Easy Linear Salt Gradient for User-Friendly Method Development:** An easy linear salt gradient method was used as an excellent starting point for method development and provided a user-friendly and flexible approach for developing analytical methods across a variety of AAV samples.
- **MALS Compatibility with Low Shedding and Accuracy:** AEX-MALS method was employed in the application for accurate quantitation of empty and full capsids with low shedding and baseline noise.
- **Monosized Base Beads:** Precision-engineered monosized base beads provide excellent higher resolving power and improved column packing and lot-to-lot reproducibility for consistent and reliable performance techniques.
- **Low Pressure with High-Pressure Limit for Fast Run:** The beads have low pressure with a high-pressure limit, enabling up to 1 mL/min high throughput fast assay.
- **Robust Lot-to-Lot Consistency:** AAV8 sample combined with UV-MALS with stringent specification for batch quality control ensures robust lot-to-lot consistency for product release.



Experimental



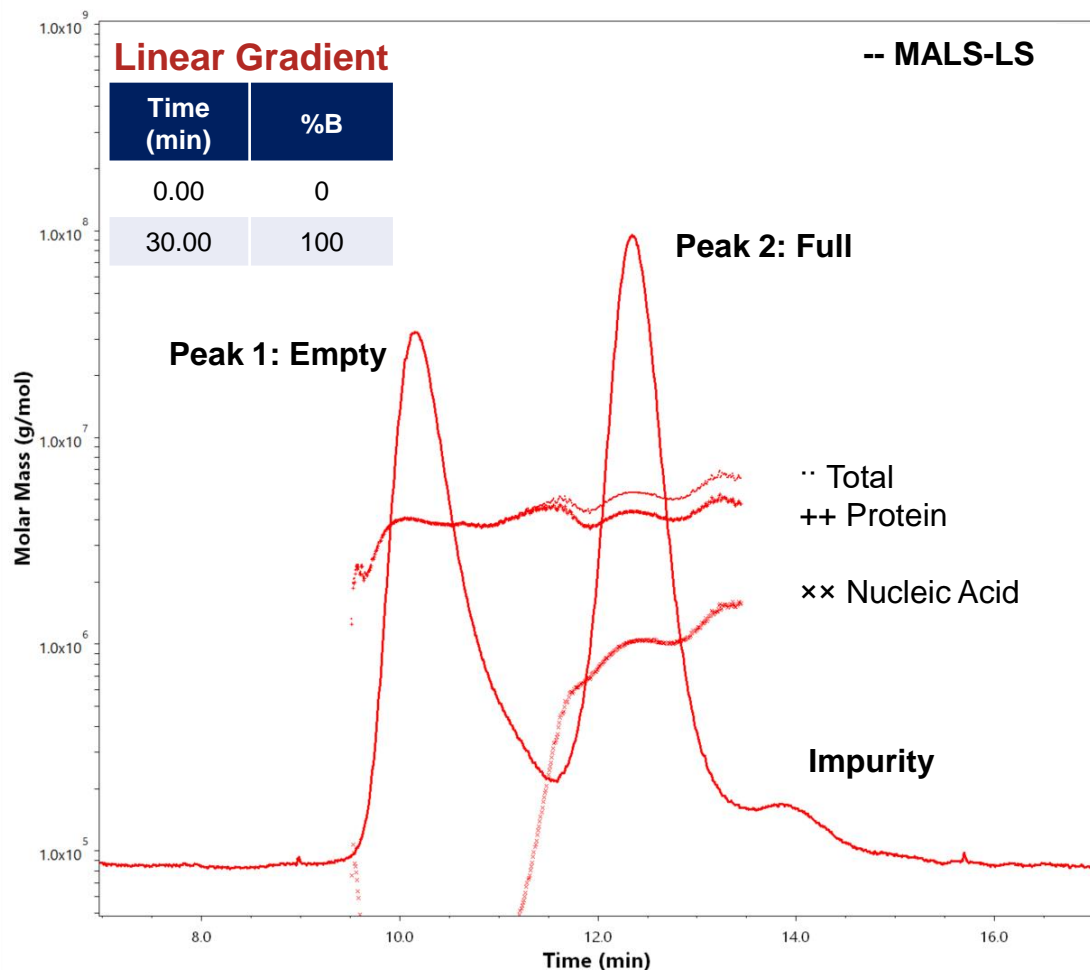
Product	<i>Proteomix</i> [®] AAV SAX
Part Number	411303P-4605
Column Dimension	4.6 × 50 mm, PEEK
Mobile Phase	Mobile Phase A: 20 mM BTP, pH=9.0 Mobile Phase B: 20 mM BTP + 500 mM TMAC, pH=9.0
Flow Rate	1.00 mL/min
Gradient	0-100 %B in 30 mins
Column Temperature	25 °C
Instrument	HPLC
Detector	MALS + UV 260, 280 nm
Sample	AAV8



AAV8 Empty and Full Capsid Separation



Full : Empty = 1:1 - On Proteomix® AAV SAX Column with UV-MALS



Sample: 2E+13 capsid/mL AAV8 (Full : Empty = 1:1); **Injection Volume:** 10 µL;
Injection Amount: 2E+11 capsid; **Pressure:** 232 bar

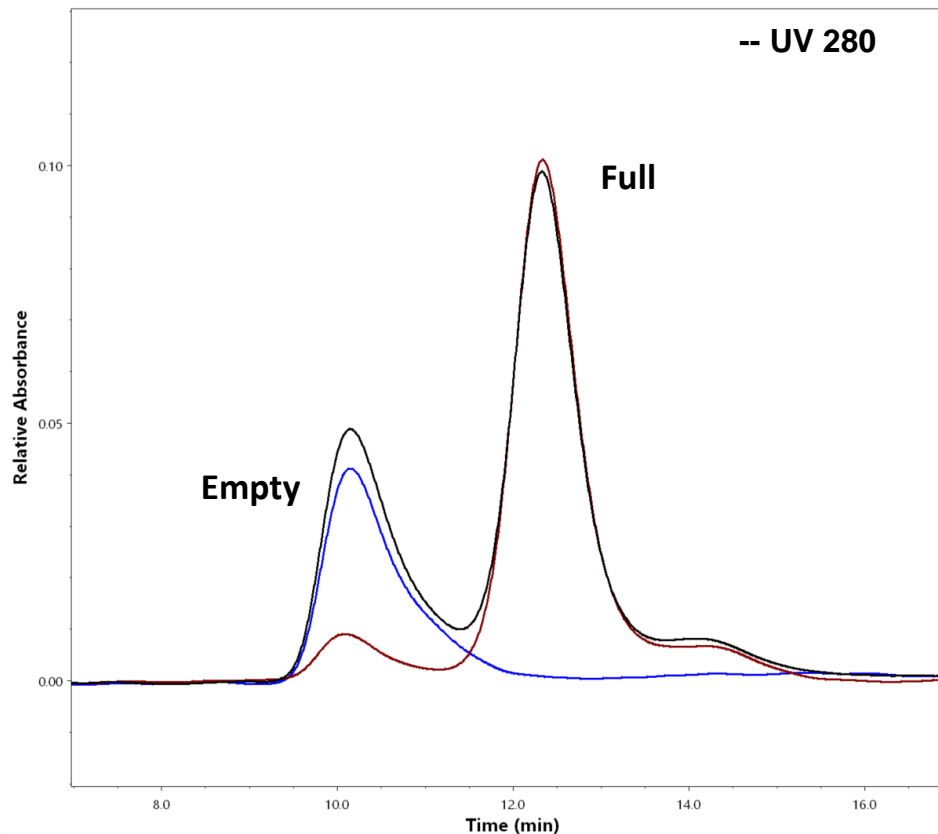
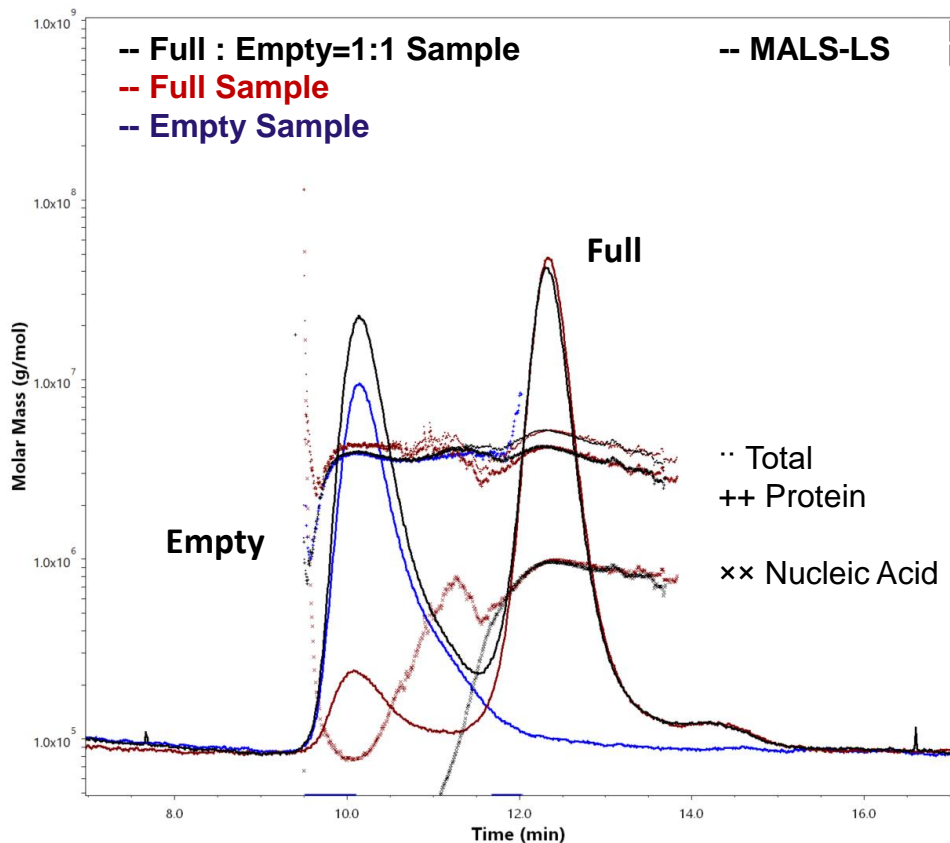
Column	Peak 1	Peak 2
Total (particles/mL)	1.1824×10^{13}	8.5209×10^{12}
Full (particles/mL)	3.3955×10^{11}	9.1612×10^{12}
Empty (particles/mL)	1.1484×10^{13}	n/a
Full to Total Ratio (Vg/Cp)	0.029	1.075
UV 280 nm Peak Area (Au min)	1.486×10^{-3}	2.891×10^{-3}
UV 260 nm Peak Area (Au min)	9.644×10^{-4}	3.777×10^{-3}
UV 260/280 Ratio	0.649	1.306
Nucleic Acid Molar Mass (g/mol)	-	$8.482 \times 10^5 (\pm 8.544\%)$
AAV8 Protein Molar Mass (g/mol)	$3.616 \times 10^6 (\pm 3.951\%)$	$3.823 \times 10^6 (\pm 3.153\%)$
Total Molar Mass (g/mol)	$3.637 \times 10^6 (\pm 3.664\%)$	$4.671 \times 10^6 (\pm 2.582\%)$



AAV8 Empty and Full Capsid Separation



Overlays of Control Standards & Full : Empty = 1:1 Mixture



Time (min)	%B
0.00	0
30.00	100

Full Empty Mixture Sample:
 2E+13 capsid/mL AAV8 (Full : Empty = 1:1);
 Injection Volume: 10 µl;
 Injection Amount: 2E+11 capsid;

Full Sample: 1E+13 capsid/mL AAV8;
 Injection Volume: 5 µl;
 Injection Amount: 1E+11 capsid;

Empty Sample: 1E+13 capsid/mL AAV8;
 Injection Volume: 5 µl;
 Injection Amount: 1E+11 capsid.

Pressure: 238 bar.

	Full : Empty=1:1 Sample		Empty Control Sample		Full Control Sample	
	Empty	Full	Empty	Full	Empty*	Full
Nucleic Acid Molar Mass (g/mol)	-	8.532×10 ⁵ (±10.170%)	-	-	-	8.805×10 ⁵ (±9.490%)
AAV8 Protein Molar Mass (g/mol)	3.677×10 ⁶ (±3.362%)	3.890×10 ⁶ (±4.357%)	3.597×10 ⁶ (±5.048%)	-	4.065×10 ⁶ (±12.999%)	3.817×10 ⁶ (±3.444%)
Total Molar Mass (g/mol)	3.700×10 ⁶ (±3.029%)	4.744×10 ⁶ (±3.816%)	3.598×10 ⁶ (±4.550%)	-	4.208×10 ⁶ (±10.590%)	4.698×10 ⁶ (±2.675%)

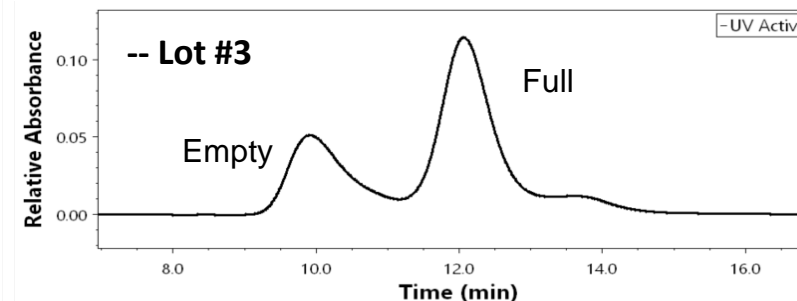
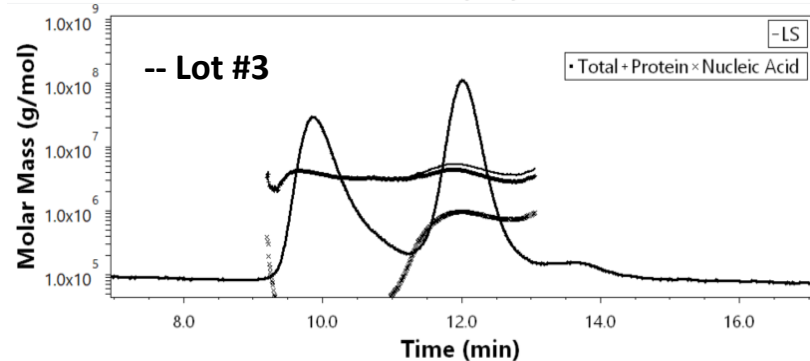
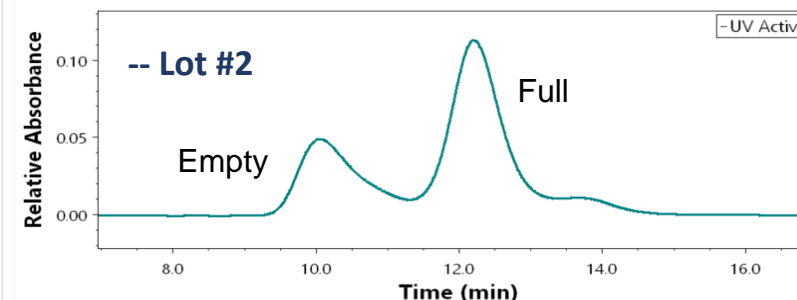
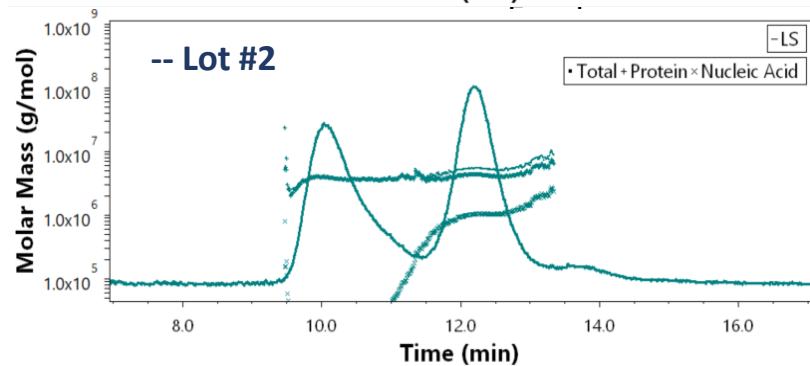
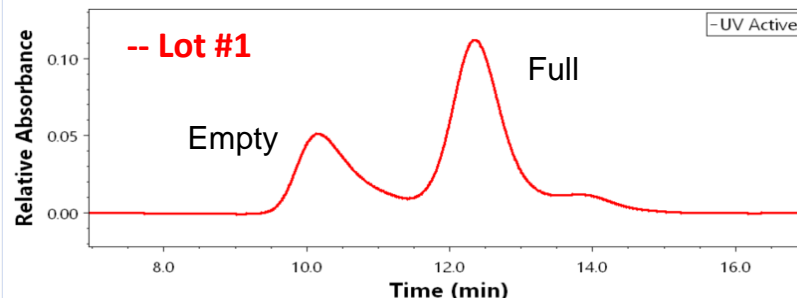
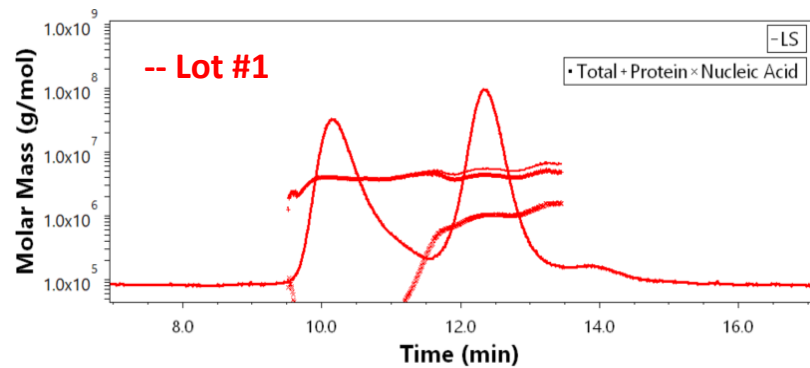
*The full AAV8 capsid-controlled sample sourced from the commercially available manufacturer may contain empty or partial capsids.



AAV8 Empty and Full Capsid Separation



Lot-to-Lot Consistency - Proteomix® AAV SAX Column



Time (min)	%B
0.00	0
30.00	100

Sample: 2E+13 capsid/mL AAV8
 (Full : Empty = 1:1);
 Injection Volume: 10 µl;
 Injection Amount:
 2E+11 capsid

Lot 1	Empty	Full
Empty (particles/mL)	1.2551×10 ¹³	n/a
Full to Total Ratio (Vg/Cp)	0.029	1.116
UV 260/280 Ratio	0.649	1.313

Lot 2	Empty	Full
Empty (particles/mL)	1.2208×10 ¹³	n/a
Full to Total Ratio (Vg/Cp)	0.032	1.146
UV 260/280 Ratio	0.654	1.322

Lot 3	Empty	Full
Empty (particles/mL)	1.2781×10 ¹³	n/a
Full to Total Ratio (Vg/Cp)	0.024	1.061
UV 260/280 Ratio	0.644	1.303



Order Information



Proteomix[®] AAV SAX

Part Number	Particle Size	Pore Size	ID × Length	Hardware
411303P-4605	3 μm	Non-Porous	4.6 × 50 mm	PEEK
411303P-4610	3 μm	Non-Porous	4.6 × 100 mm	PEEK

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For Technical Questions & Method Development: techsupport@sepax-tech.com

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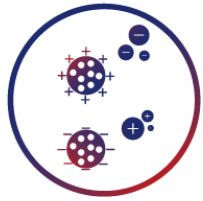
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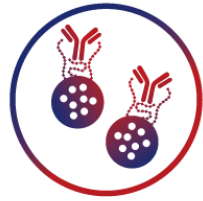
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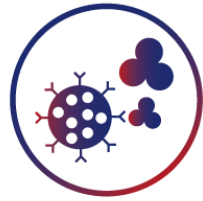
Sepax Products



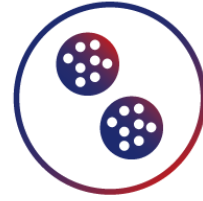
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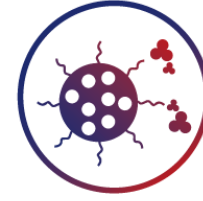
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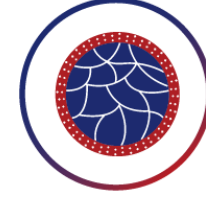
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SEC



RP

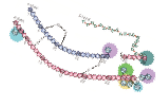


Mixed-mode

Full Life Cycle Support of Analytical and Process Chromatography



Bispecific



Insulin



Plasmid



Peptides

ADC

mRNA



mAb



Recombinant Protein



AAV



Adenovirus

Vaccine