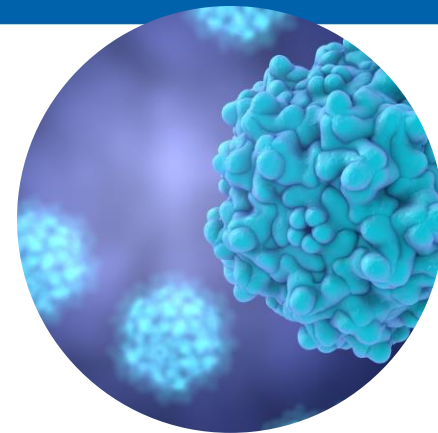




Detector Versatility for Adeno-Associated Virus (AAV) Aggregate Analysis with a Biozen™ dSEC-7 Column

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Overview

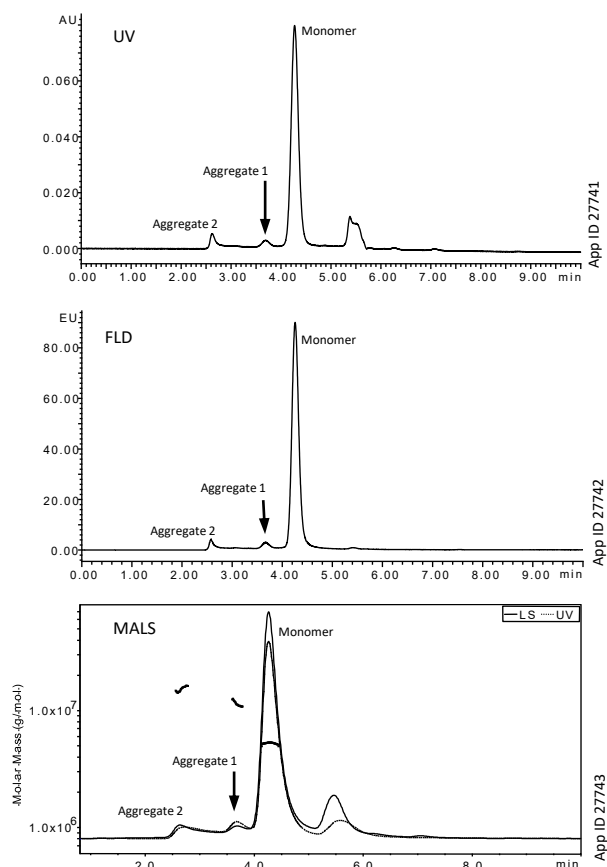
Size Exclusion Chromatography (SEC) stands out as a reliable technique for AAV aggregation measurement. While a UV detector is commonplace for SEC analysis, the conventional SEC-UV approach demands substantial sample amounts for sensitive readings and provides limited information beyond aggregate quantitation. For this reason, other modes of detection may be utilized as they offer advantages over UV. SEC-FLD is often adopted for its heightened sensitivity which is crucial to accommodate the low concentrations produced from the low product yields of AAV manufacturing. Alternatively, SEC-MALS eliminates the need for multiple assays, offers superior precision and accuracy, and its ability to monitor deviations in protein molar mass offers deeper insight into potential issues such as incorrect assembly, aggregation, fragmentation, or degradation.

This application note showcases the Biozen dSEC-7 column's performance in minimizing sample consumption without compromising data quality in aggregate analysis in multiple AAV serotypes, particularly when employing SEC-Fluorescence (SEC-FLD). Additionally, the combination of the Biozen dSEC-7 column with SEC-MALS enables thorough aggregate analysis and precise determination of molecular weights (both monomer and aggregates).

LC Conditions

Column: Biozen 3 μ m dSEC-7
Dimensions: 150 x 4.6 mm
Part No.: [00F-4789-E0](#)
Mobile Phases: 20 mM Sodium Phosphate, pH 6.6 + 350 mM Potassium Chloride
Flow Rate: 350 μ L/min
Injection Volume: 20 μ L (LC-UV)
2 μ L (LC-FLD)
20 μ L (LC-MALS)
Temperature: 25 $^{\circ}$ C
Instruments: Waters® ACQUITY® H-Class Bio, Quaternary Pump
Detection: UV @ 280 nm
FLD @ Ex: 280 nm, Em: 350 nm
MALS @ 280 nm
Samples: 1. AAV2-CAG-GFP, 2E13 vg/mL
2. AAV5-CMV-GFP, 2E13 vg/mL
3. AAV9-CMV-GFP, 2E13 vg/mL

Figure 1. AAV2 Monomer and Aggregate Separation Across Different Detectors Using a Biozen 3 μ m dSEC-7 Column.



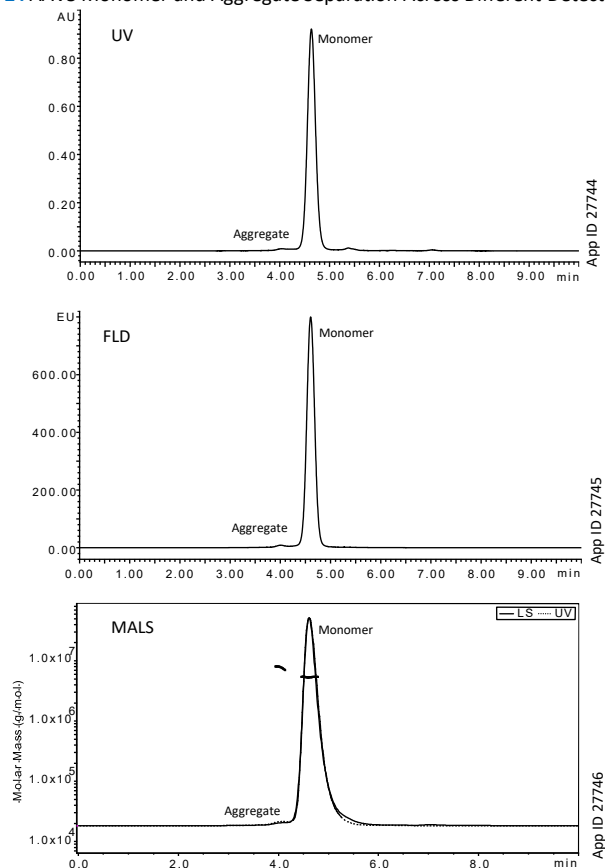
UV				
Peak	Retention Time (min)	% Area	Resolution	
Aggregate 2	2.62	4.39	-	4.74
Aggregate 1	3.69	2.80	2.11	
Monomer	4.27	92.80	-	-

FLD				
Peak	Retention Time (min)	% Area	Resolution	
Aggregate 2	2.58	2.77	-	5.32
Aggregate 1	3.68	2.53	2.28	
Monomer	4.26	94.69	-	-

MALS				
Peak	Retention Time (min)	% Mass Fraction	Resolution	MW (kDa)
Aggregate 2	2.58	1.90	-	5.32
Aggregate 1	3.68	2.80	2.28	
Monomer	4.26	95.30	-	5238.5 ($\pm$ 21.8 %)



Figure 2 . AAV5 Monomer and Aggregate Separation Across Different Detectors Using a Biozen™ 3 µm dSEC-7 Column.

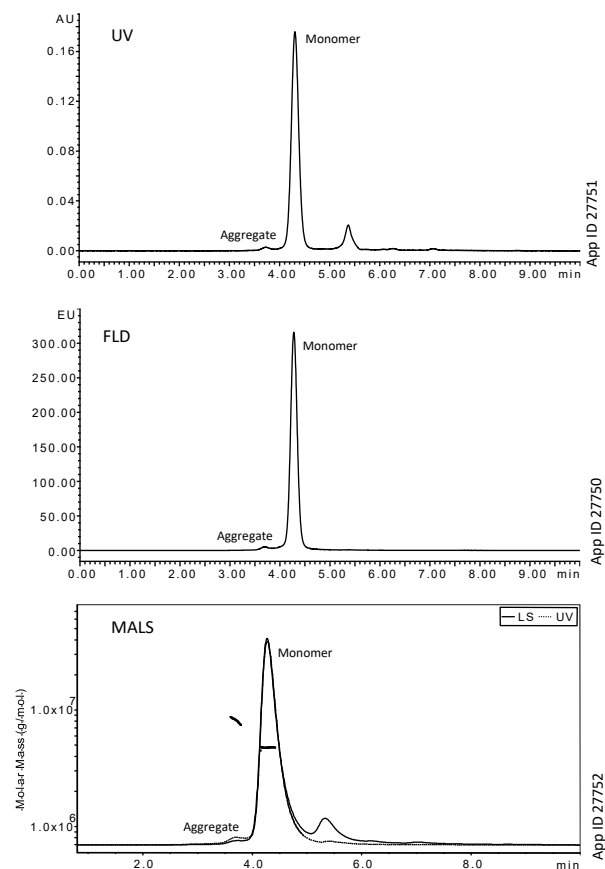


UV			
Peak	Retention Time (min)	% Area	Resolution
Aggregate	4.04	0.82	2.0
Monomer	4.64	97.67	

FLD			
Peak	Retention Time (min)	% Area	Resolution
Aggregate	3.99	0.58	2.08
Monomer	4.60	99.42	

MALS				
Peak	Retention Time (min)	% Mass Fraction	Resolution	MW (kDa)
Aggregate	4.04	1.00	1.90	7689.5 (±21.7 %)
Monomer	4.64	99.00		5347.5 (±21.9 %)

Figure 3 . AAV9 Monomer and Aggregate Separation Across Different Detectors Using a Biozen 3 µm dSEC-7 Column.



UV			
Peak	Retention Time (min)	% Area	Resolution
Aggregate	3.71	1.46	1.86
Monomer	4.31	98.54	

FLD			
Peak	Retention Time (min)	% Area	Resolution
Aggregate	3.69	0.93	2.28
Monomer	4.28	99.07	

MALS				
Peak	Retention Time (min)	% Mass Fraction	Resolution	MW (kDa)
Aggregate	3.71	1.70	1.86	8081.0 (±18.8 %)
Monomer	4.31	98.30		4740.2 (±18.9 %)



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