

Wide-pore analytical SEC-HPLC of AAV8 and AAV9 with Sepax SRT SEC-1000

Mechanistic Studies and Formulation Mitigations of Adeno-associated Virus Capsid Rupture During Freezing and Thawing: Mechanisms of Freeze/Thaw Induced AAV Rupture¹

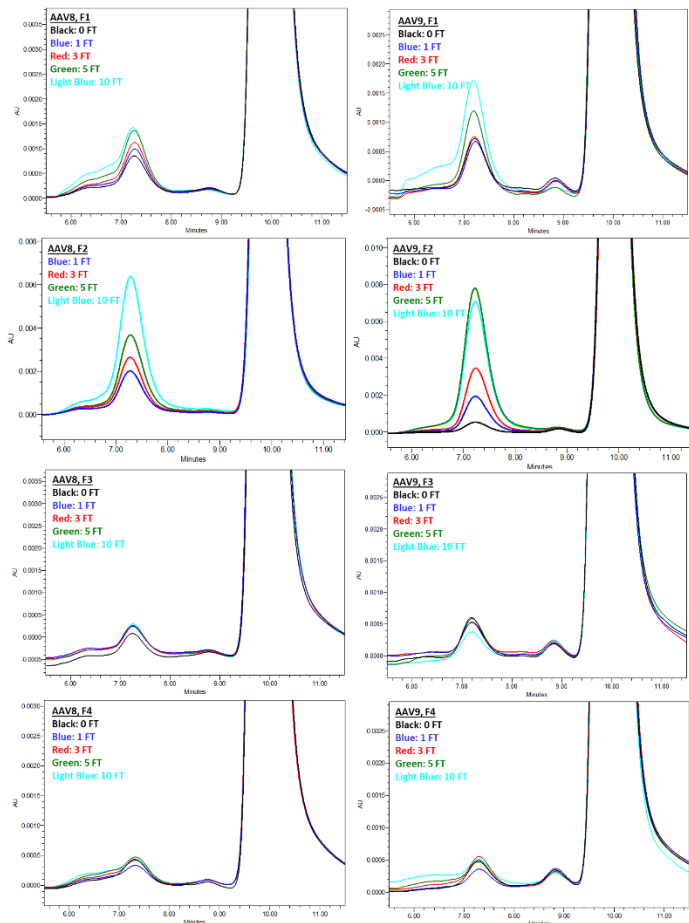


Figure S2 Size exclusion chromatography profiles at 260 nm for AAV8 (left panels) and AAV9 (right panels) subjected to F/T cycles in formulations F1, F2, F3, and F4.

HPLC Column	Sepax, SRT SEC-1000 PEEK, 5um, 1000 A 4.6 x 300 mm Part Number: 215950P-4630
Mobile Phase	20 mM sodium phosphate, 300 mM sodium chloride, 0.005% w/v poloxamer 188, pH 6.5
Flow Rate	0.35 mL/min
Instrument	UVSEC-MALS; SLS - Static Light Scattering
Sample	AAV8 and AAV9

Rupture of AAV capsids during F/T cycles can be studied with size exclusion chromatography (SEC). Sepax SRT SEC-1000, separates aggregates and free DNA from intact adeno-associated virus capsids.

- ❑ Pre-peak 1&2 (~8.7 and 8.0 min) are assigned as aggregated species based on A260/280 ratios
- ❑ Pre-peak 3 (~7.2 min) is assigned as free DNA with no protein present since the A260/A280 ratio is ~1.8. It elutes before the capsid since it has a larger effective hydrodynamic size when released from its tightly packed state inside the capsid
- ❑ Monomer peak of AAV capsid

“Addition of $\geq 0.0005\%$ w/v poloxamer 188 (P188) eliminated substantial recovery losses (up to ~60% without P188) and minimized rupture to $\leq 1\%$ per F/T cycle (as indicated by SEC-HPLC)”

Part Number: [215950P-4630](#)



REGENXBIO Inc.

[1] Bee, Jared S., et al. "Mechanistic Studies and Formulation Mitigations of Adeno-Associated Virus Capsid Rupture during Freezing and Thawing: Mechanisms of Freeze/Thaw Induced AAV Rupture." *Journal of Pharmaceutical Sciences* (2022).