



TOSOH

## LENS<sup>TM</sup><sub>3</sub> MALS DETECTOR

Multi-Angle Light Scattering  
made easy.

Powerful.

Versatile.

Accessible.



TOSOH BIOSCIENCE  
**SEPARATION  
& PURIFICATION**

CONNECTING MINDS.  
TOUCHING LIVES.

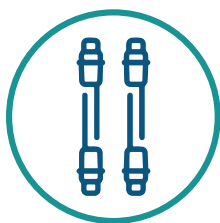
## Comprehensive Solution for Advanced Gel Permeation/Size Exclusion Chromatography

Biopharmaceutical macromolecules such as proteins and antibodies, viral or polymer-based drug delivery vectors, new sustainable polymers and common engineering plastics all require increasingly thorough characterization to develop well-defined and safe products. With over 50 years of experience in chromatography, Tosoh Bioscience provides a complete solution to support your analysis needs.



### LenS<sup>TM</sup><sub>3</sub> Multi-Angle Light Scattering Detectors

The LenS<sub>3</sub> MALS detector series offers an advanced approach for the measurement of molecular weight (MW) and size distributions of delivery vectors, biomolecules, synthetic polymers, polysaccharides, and biopolymers. Powered by simple, powerful and accurate SECview<sup>TM</sup> software.



### TSKgel<sup>®</sup> GPC/SEC Columns

Extensively used in laboratories all over the world, our TSKgel columns are designed for researchers seeking the highest level of performance. Covering the total range of GPC/SEC applications with multiple packing materials, porosities, and dimensions, these columns offer high resolution, excellent reproducibility and long column life.



### EcoSEC<sup>TM</sup> GPC Systems

The EcoSEC series of fully automated liquid chromatography systems for gel permeation chromatography is designed for robust polymer analysis. Both solutions, for ambient and for high temperature GPC, combine dual pump solvent systems, sophisticated heating and a highly efficient refractometry detection system to deliver the highest reproducibility.

## Get Started

Additional resources are available for helping you implement the LenS<sub>3</sub> Multi-Angle Light Scattering Detector into your laboratory.



### Web

Visit [tosohbioscience.com](http://tosohbioscience.com) for videos, product information and ordering.



### Email

Our technical service staff is ready to answer questions:  
*In the Americas:*  
[techservice.tbl@tosoh.com](mailto:techservice.tbl@tosoh.com)  
*In the EMEA:*  
[techsupport.tb@tosoh.com](mailto:techsupport.tb@tosoh.com)



### In Person

A technical seminar can be arranged on-site or via the web:  
*In the Americas:*  
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## LenS<sub>3</sub> Multi-Angle Light Scattering Detector

*Versatility, performance and accessibility for the characterization of polymers and biomolecules*

Introduced in 2019, the LenS<sub>3</sub> MALS detector featured the first major technological advancements in light scattering instrumentation in over four decades. Powered by the SECview software, Tosoh's GPC/SEC-MALS solution has been the perfect complement to our renowned expertise in size exclusion chromatography technologies.

Building upon the unique design of the original LenS<sub>3</sub> MALS detector and SECview software, the new generation enhances the benefits of MALS by making analyses even more accessible, intuitive and reliable, while offering the widest versatility and highest performance.

Measuring molecular weight (MW), radius of gyration ( $R_g$ ), and much more of polymers and biomolecules has never been so seamlessly achievable.

➤ **Figure 1.** Main features and benefits of the LenS<sub>3</sub> MALS solution

### Unique patent-pending flow cell and optics design

- Extended MW and  $R_g$  measurement range
- High sensitivity for low-scattering samples (low MW, low concentration, low  $dn/dc$ )

### Adjustable laser power

- Allows measurement of both low and high-scattering samples (high concentration or MW)

### Low volume inert flow cell

- Use with any organic or aqueous mobile phase
- Compatible with HPLC/UHPLC systems and columns
- No loss of chromatographic resolution

### Intuitive software and workflow with powerful data processing

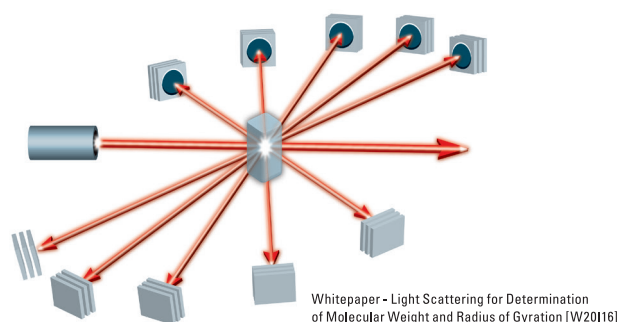
- Easy to learn, use and teach
- All results in a few clicks with no model assumption
- High productivity

## Advanced Detector Design

Multi-Angle Light Scattering (MALS) detectors have become a common tool to determine MW and size of macromolecules (Figure 2). The theory is based on the Rayleigh equation, where the intensity of the scattered light,  $R_{\theta}$ , is directly related to molecular weight of macromolecules. Historically, there have been two approaches to obtaining MW and size from light scattering measurements.

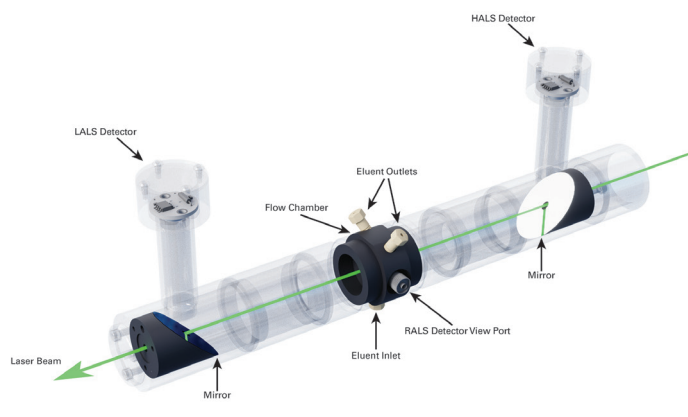
Using the Zimm method, MW weight and  $R_g$  of large scattering molecules can be determined on the basis of angular and concentration dependence measurements of the intensity of scattered light from dilute solutions. Alternatively, MW can be obtained accurately from a Low Angle Light Scattering (LALS) detector directly without angular extrapolation.

➤ Figure 2. Typical MALS detector design



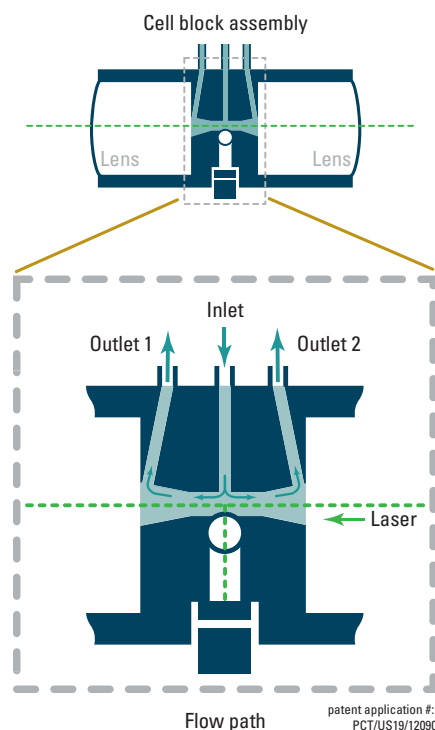
The LenS<sub>3</sub> Multi-Angle Light Scattering detector is a technological advancement that combines the best of both MALS and LALS detectors. It does not contain a conventional cell and offers an extended flow path that uses 3 angles to provide MALS and LALS analysis, as depicted in Figure 3.

➤ Figure 3. LenS<sub>3</sub> MALS detector design

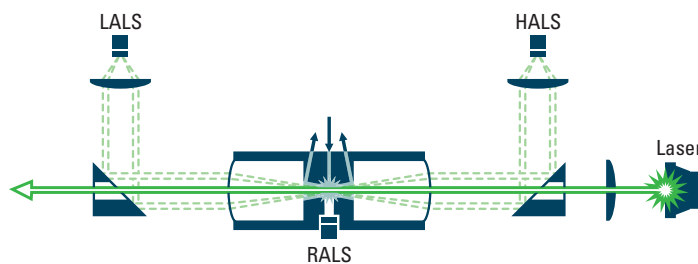


The angles are fixed at 10° (LALS), 90° (RALS) and 170° (HALS), while the inlet flow is split into two at the entrance of the measurement path and exits at two separate outlets. Additionally, a green laser ( $\lambda = 505$  nm) provides approximately 2.7 times higher scattering intensity than a conventional red laser ( $\lambda = 660$  nm). Greater sensitivity is also provided by the unique design of the light path, as opposed to a conventional flow cell, which allows maximum interaction with solute molecules and a more effective light collection mechanism with lower noise (see Figures 4 and 5).

➤ Figure 4. Cell block assembly and flow path of the LenS<sub>3</sub> MALS detector



➤ Figure 5. Optics design of LenS<sub>3</sub> MALS detector



## Versatile Calculation Methods

The LenS3 MALS detector's number and the positions of the detector angles enable a unique and unmatched capability to offer users multiple calculation options for MW and  $R_g$  via the SECview software.

### MW and $R_g$ determination options:

Molecular Weight	Radius of gyration
<b>Direct measurement using LALS (10°)</b> – Optimal and accurate for any applications across the most common MW range of polymers	<b>Angular Dissymmetry Plot*</b> – Optimal and accurate for $R_g$ up to 50 nm
<b>Direct measurement using RALS (90°)</b> – Perfect for most biomolecules and small polymers, for which S/N ratio matters even more	
<b>Combined Conformation Model**</b> – Recommended for ultra-high MW large polymers and biomolecules ( $R_g > 50$ nm), regardless of their structure and conformation	
Historical multi-angle measurements and extrapolations (Zimm, Debye, Berry, Guinier) – For comparison with legacy methods	

\* PCT/US19/12095: Light Scattering Detectors and Methods for the Same

\*\* WO 2023/038621 A1: Light Scattering Detectors and Methods for the Same

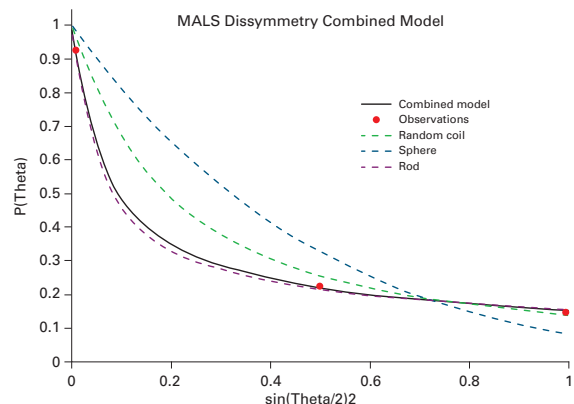
\* patent application #: PCT/US19/12095: Light Scattering Detectors and Methods for the Same

Whitepaper - Light Scattering for Determination of Molecular Weight and Radius of Gyration [W20116]

Expanding the boundaries of light scattering [TN21107A]

The patented Combined Conformation Model determination of  $R_g$  and MW computes the combination of three conformation models (spherical, random coil and rod-like) and provides the resulting scattering pattern that fits the actual scattered light at the three observation angles (10°, 90° and 170°).

Figure 6. Angular dissymmetry patterns of models and combined best fit



This proprietary method benefits from the unique design of the LenS3 MALS detector, specifically the lower wavelength light source and the extreme low and high angles.





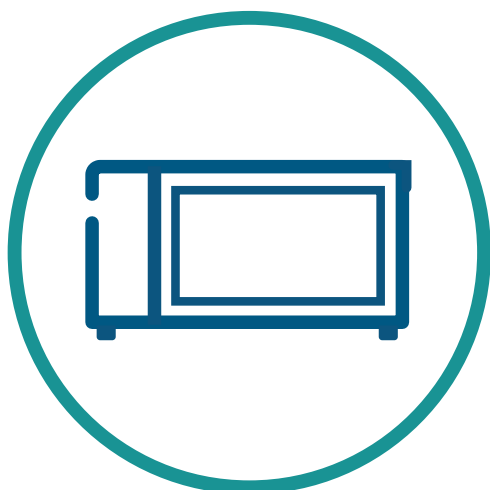
SECview is 21-CFR-Part 11 compliant and provides the necessary tools for audit trails, system validation and multi-user access for users operating in the FDA-regulated environment.

### SECview Features

- Semi-automated peak integration
- Multi-file data processing
- Simultaneous execution of multiple analysis methods
- Multi-point  $dn/dc$  and UV extinction coefficient determination
- Automatic peak detection for legacy column calibration methods
- Multi-peak selection and data processing
- Adaptable multiple-injection overlay platform
- Advanced band broadening and inter-detector volume correction algorithms
- Direct access to the raw data signals while offering powerful de-spiking options
- Access to slice-by-slice data on chromatograms and distribution graphs
- Easy export of raw and calculated data

### SECview Parameters

- True molecular weight by light scattering, in bulk and distribution ( $PDI$ ) forms
- Radius of gyration by MALS and conformation plots
- Molecular weight by column calibration
- Concentration, injection-mass recovery,  $dn/dc$ , and UV extinction coefficient
- Composition analysis of conjugates and copolymers
- Branching analysis of polymers
- Empty/full ratio and particle count of AAVs



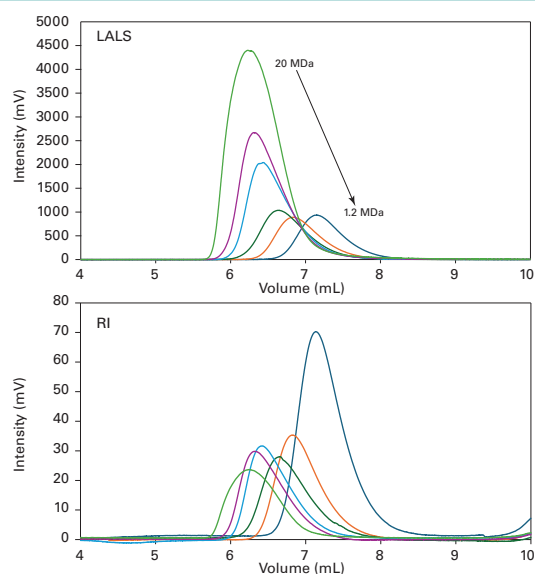
## Accurate Molecular Weight and $R_g$ Determination

**Molecular weight and radius of gyration are measured accurately across the widest range of polymer size.**

### System & Columns

- EcoSEC Elite GPC system with RI detector
- LenS3 MALS detector
- 1 x TSKgel® GMH<sub>HR</sub>-M or GMH<sub>HR</sub>-H (7.8 mm ID x 30 cm) column

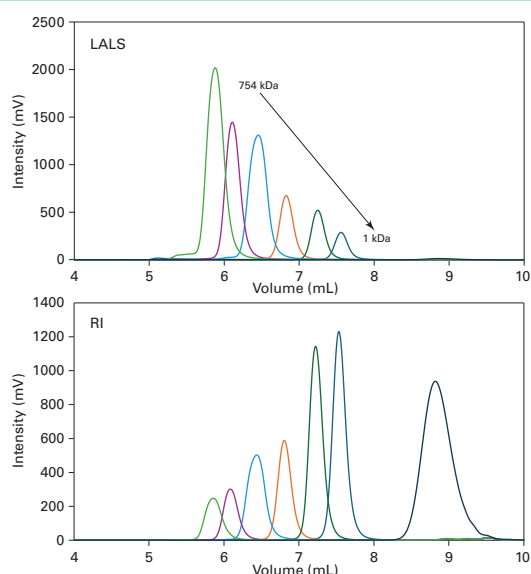
➤ **Figure 7.** LALS (10°) and RI signals of ultra-high MW PS standards



### Samples & Conditions

- Tosoh polystyrene (PS) standards
- THF at 1.0 or 0.5 mL/min, T=40°C (pumps, columns and RI)
- Concentration = 0.2 to 2.5 mg/mL; Injection Volume = 50 µL

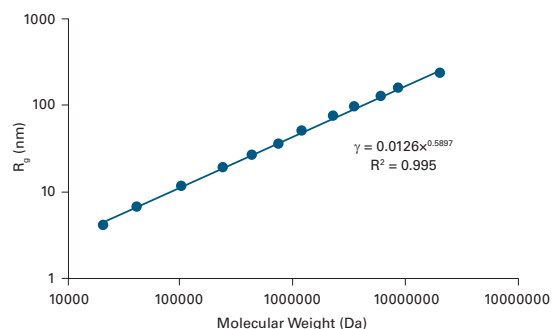
➤ **Figure 8.** LALS (10°) and RI signals of medium to low MW PS standards



➤ **Table 1.** Measured values of MW and  $R_g$  of PS standards by MALS

PS standard	MW (Da)	$R_g$ (nm)
A-1000	1,050	-
F-1	10,800	-
F-2	21,000	4.2
F-4	41,030	6.6
F-10	103,500	11.8
F-20	237,100	19.1
F-40	431,500	26.4
F-80	745,600	35.4
F-128	1,214,600	51.0
F-288	2,279,000	73.5
F-380	3,476,000	95.3
F-550	5,981,500	128
F-850	8,407,000	153
F-2000	20,077,000	233

➤ **Figure 9.** Conformation plot obtained from Polystyrene standards is in agreement with literature data (J. Mays *et al.*, Journal of Applied Polymer Science, Vol. 61, 865-874 (1996))



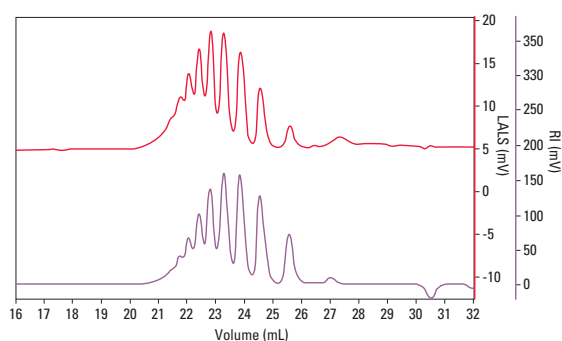
## Ultra-Low Molecular Weight Oligomers by LALS

### The Low Angle Light Scattering detector is capable of measuring molecular weight down to 200 Da!

#### System & Columns

- EcoSEC GPC System with RI detector
- LenS3 Multi-Angle Light Scattering detector
- 1 × G3000HXL, 1 × G2500HXL and 1 × G2000HXL (all 7.8 mm ID × 30 cm) columns

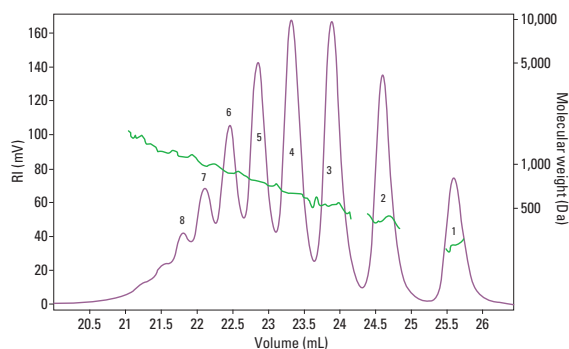
➤ **Figure 10.** LALS (red) and RI (purple) signals of the A-500 PS standard



#### Samples & Conditions

- Tosoh A-500 polystyrene (PS) standard
- THF at 1.0 mL/min; T = 35 °C (pumps, column and RI)
- Concentration = 8.54 mg/mL; injection volume = 50 µL

➤ **Figure 11.** Molecular weight distribution (green) overlaid with RI signal



➤ **Table 2.** Theoretical molecular weights of the oligomers present in A-500 and  $M_p$  values obtained from LALS (using an average  $dn/dc$  of 0.170)

Peak #	Theoretical MW	$M_p$ from LALS
1	266	255
2	370	377
3	474	503
4	578	618
5	682	725
6	786	849
7	890	985
8	994	1105

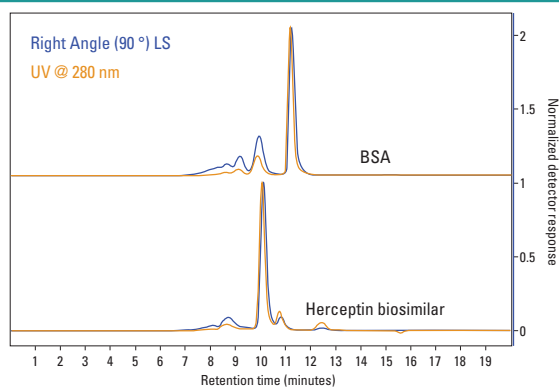
## Protein Aggregates and Fragments Identification and Quantitation

### Oligomers and fragments of BSA and monoclonal antibodies are easily detected and identified with the LenS<sub>3</sub> MALS detector.

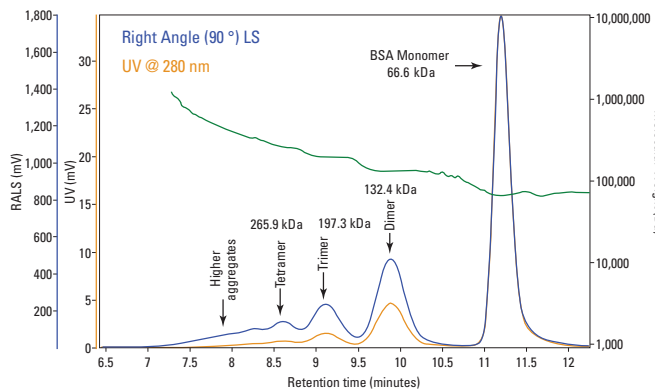
#### System & Columns

- Thermo Fisher Dionex Ultimate® 3000 UHPLC system with UV detector @ 280 nm
- LenS<sub>3</sub> Multi-Angle Light Scattering detector
- 1 x TSKgel UP-SW3000 (4.6 mm ID x 30 cm) column

➤ **Figure 12.** Raw chromatograms of BSA and Herceptin biosimilar samples, with light scattering and UV signals



➤ **Figure 13A.** Molecular weight (green) distribution curve and values of BSA oligomers overlaid with UV (orange) and RALS (blue) signals

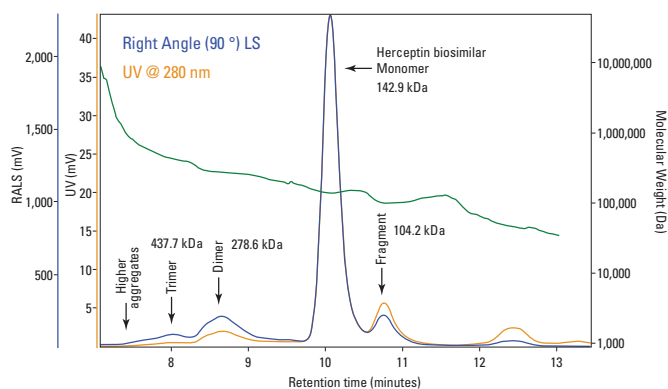


Peak	MW (kDa)	Area (%)
Monomer	66.6	74.1
Dimer	132.4	16.7
Trimer	197.3	5.4
Tetramer	265.9	2.1
Aggregates	Up to 1,000+	1.7

#### Samples & Conditions

- Bovine Serum Albumin (BSA) and Herceptin® biosimilar
- 100 mmol/L NaH<sub>2</sub>PO<sub>4</sub>, pH 6.8 + 100 mmol/L Na<sub>2</sub>SO<sub>4</sub>
- Flow rate = 0.25 mL/min
- Concentration:  
BSA = 3.58 mg/mL, injection volume = 10 µL  
mAb = 2.75 mg/mL, injection volume = 7 µL

➤ **Figure 13 B.** Molecular weight (green) distribution curve and values of Herceptin biosimilar overlaid with UV (orange) and RALS (blue) signals



Peak	MW (kDa)	Area (%)
Fragment	104.2	11.8
Monomer	142.9	69.1
Dimer	278.6	8.3
Trimer	437.7	1.3
Aggregates	Up to 5,000+	0.3

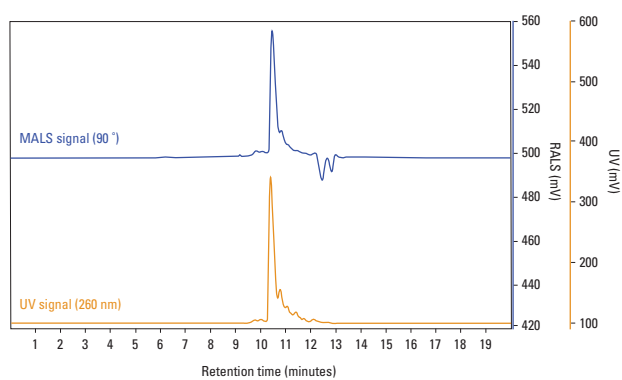
## Analysis of Unpurified and Purified Oligonucleotides

### Rapid and accurate molecular weight profiling of small oligonucleotides is now possible!

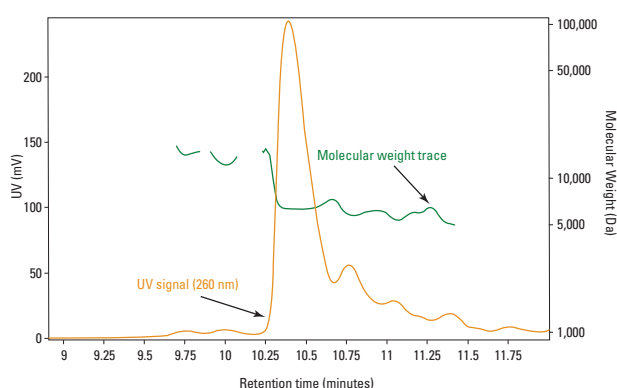
#### System & Columns

- Thermo Fisher Dionex Ultimate 3000 UHPLC system with UV detector @ 260 nm
- LenS3 Multi-Angle Light Scattering detector
- 1 × TSKgel UP-SW2000 (4.6 mm ID × 30 cm) column

➤ **Figure 14.** RALS (blue) and UV (orange) signals of the unpurified 20-mer



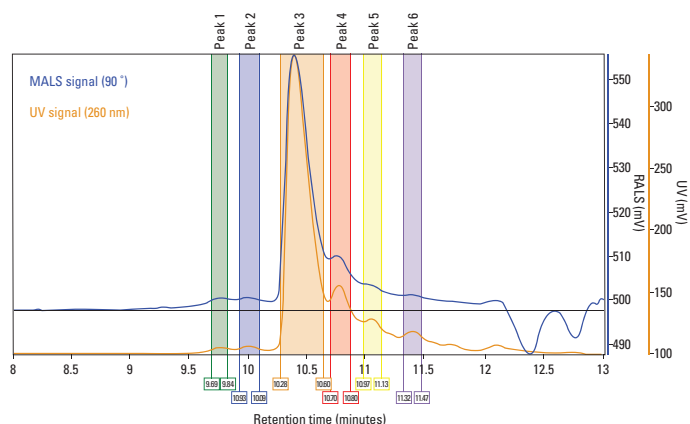
➤ **Figure 15.** Molecular weight distribution (green) of the unpurified 20-mer



#### Samples & Conditions

- 20-bases custom oligonucleotide with MW=6,141 Da
- 0.5 mol/L NaCl + 0.1 mol/L EDTA + 0.1 mol/L Na<sub>2</sub>SO<sub>4</sub> + 0.05% NaN<sub>3</sub> in 0.1 mol/L phosphate buffer, pH 7.52
- Flow rate = 0.30 mL/min
- Injection volume = 10 µL
- Concentration:  
Purified sample = 0.3 mg/mL  
Unpurified sample = 1 mg/mL

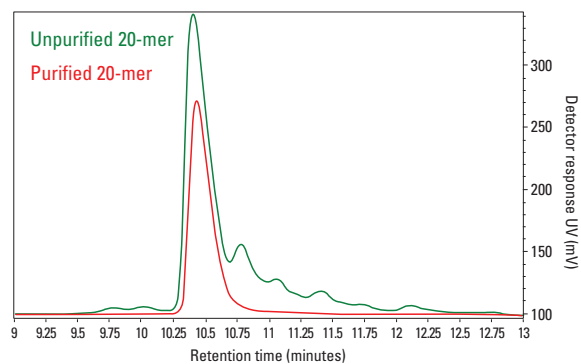
➤ **Figure 16.** Peak analysis of the unpurified 20-mer



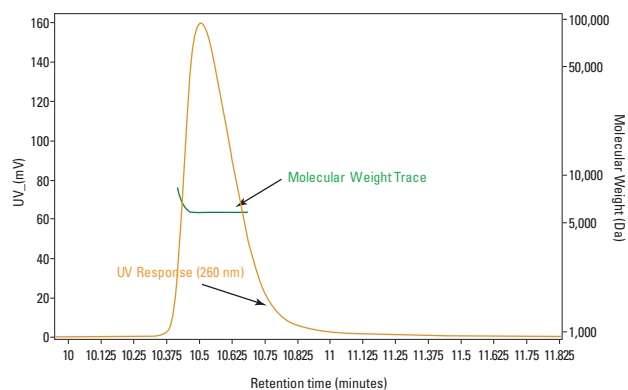
➤ **Table 3.** Retention time and molecular weight of each peak (triplicate injection)

Peak	Retention time (min)	% RSD	MW (Da)	% RSD
1	9.774	0.1%	13,599	2.1%
2	10.012	0.0%	11,550	1.9%
3	10.398	0.1%	6,398	0.7%
4	10.776	0.1%	5,751	1.5%
5	11.053	0.1%	5,177	2.3%
6	11.422	0.2%	4,446	5.5%

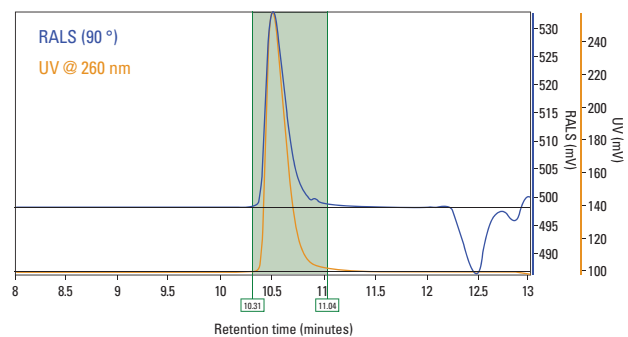
➤ **Figure 17.** Overlay of the unpurified (green) and purified (red) 20-mer UV chromatograms



➤ **Figure 18.** Molecular weight distribution (green) of the purified 20-mer



➤ **Figure 19.** Peak analysis of the purified 20-mer



➤ **Table 4.** Retention time and molecular weight of the purified 20-mer (triplicate injection)

Injection	Retention time (min)	MW (Da)
1	10.431	6,066
2	10.443	6,023
3	10.445	6,038
Average	10.440	6,042
<b>%RSD</b>	<b>0.1%</b>	<b>0.3%</b>

## Determination of empty/full ratio of Adeno Associated Viruses (AAV)

### Easily determine the empty/full ratio and titer of AAVs with the LenS<sub>3</sub> MALS detector and SECview software.

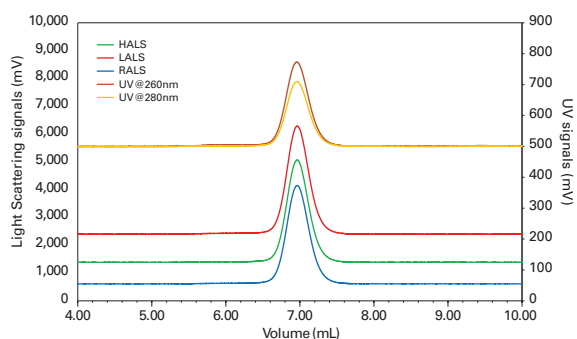
#### System & Columns

- Thermo Fisher Dionex Ultimate 3000 system with UV detector @ 280 and 260 nm
- LenS<sub>3</sub> MALS detector
- 1x TSKgel GMPW<sub>XL</sub> (7.8 mm ID × 30 cm) column

#### Samples & Conditions

- AAV5
- Phosphate buffer with 20 mmol/L sodium phosphate, 0.5 mol/L sodium chloride, pH 6.5
- Flow rate = 0.30 mL/min
- Titer =  $2 - 4 \times 10^{13}$  particles/mL

➤ **Figure 20.** Light Scattering signals (LALS (10°), RALS (90°), HALS (170°)) and UV signals (@ 260 nm and 280 nm) of a commercial AAV5 sample.



➤ **Table 6.** Known parameters of the AAV5 sample

	ssDNA payload	Capsid
Molecular Weight (Da)	792,000	3,706,000
$dA/dc$ @ 260 nm (mL/mg)	30	1.178
$dA/dc$ @ 280 nm (mL/mg)	16.667	2.0
$dn/dc$ (mL/g)	0.170	0.185

➤ **Table 5.** Critical Quality Attributes of the AAV5 sample

	AAV5
Molecular Weight (Da)	4,376,400
Titer (1/mL)	$3.93 \times 10^{13}$
Radius – $R_g$ (nm)	9.4
Empty/Full ratio (Wt% full)	84.7%

## Attributes

Light scattering	
Number of measurement angles	3
Position of the measurement angles	LALS (10°) RALS (90°) HALS (170°)
Cell geometry	Patented dual conical flow path (single inlet, dual outlets)
Total cell volume	43 µL
Laser source type	Diode
Laser power	1 – 50 mW (User adjustable)
Laser wavelength	505 nm
Laser temperature control	Peltier
Wetted material	PTFE, PEEK, glass
Maximum flow rate	5 mL/min
Inlet position	Side port (left)
Baseline noise (RMS without despiking) on RALS in THF @ 1 mL/min	< 1 mV
Typical signal-to-noise ratio on RALS for 50 µg of PS 100 KDa*	2000:1
MW range	< 200 to 10 <sup>6</sup> Da**
R <sub>g</sub> range	< 5 to > 250 nm**
Despiking level	User selectable (None, low, mid, high)

General	
Acquisition rate	10 Hz
A/D board channels / resolution	8 channels / 24 bit
Dynamic range	+/- 10 V
External analog inputs	2 auxiliary channels
Trigger input	<= 12 V DC requiring dry contact closure
Trigger output	Dry contact closure
Alert trigger output	Dry contact closure
External USB hardware	2 USB A, 1 USB C (powered)
Onboard PC OS	Windows 11 PRO
Onboard RAM / Storage	16 GB / 1 TB
Onboard processing	SECview software
PC Connection	Ethernet cat 5
Touch screen	13.3"
Dimensions	46.0 (W) × 25.1 (H) × 58.5 (D) cm = 18.1" × 9.9" × 23"
Weight	19 kg / 42 lbs
Shipping weight	23 kg / 51 lbs
Operating environment	5 – 35° Celsius @ 10 to 80% relative humidity (no condensation)
Instrument voltage	100 – 240 V AC @ 50 / 60 Hz
Power requirements	Typical 110 W, maximum 700 W
Intellectual property	PCT/US19/12090: Light Scattering Detectors and Sample Cells for the Same PCT/US19/12095: Light Scattering Detectors and Methods for the Same WO 2023/038621 A1: Light Scattering Detectors and Methods for the Same

\*Narrow distribution, using 1 × 30 cm L × 7.8 mm ID TSKgel GMH<sub>HR</sub>-M column

\*\*Depends on chromatographic separation and sample injected mass, conformation and refractive index increment ( $dn/dc$ )

## Ordering Information

Part #	Description
0040100	LenS <sub>3</sub> Multi-Angle Light Scattering Detector



**TOSOH BIOSCIENCE**

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