TOSOH

TSKgel® UP-SW3000-LS - Size Exclusion UHPLC Columns

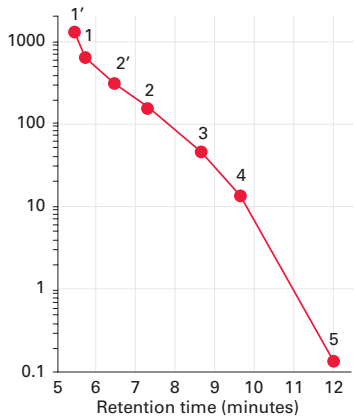
Designed for smooth and efficient combination with light scattering and mass spectrometry detection

Aqueous size exclusion chromatography (SEC) is an established method for the analysis of proteins and monoclonal antibodies, including fragments, monomers, and aggregates under non-denaturing conditions. TSKgel SW series columns have been the industry's workhorses for the SEC analysis of biotherapeutics for decades. TSKgel UP-SW3000-LS columns are the latest addition to this renowned column family.

TSKgel UP-SW3000-LS columns offer high resolution, sharp peak shape and high efficiency yielding methods that are robust, reproducible and easily transferable between UHPLC and HPLC systems. These U/HPLC columns provide significantly lower noise levels than other columns when coupled with advanced detectors, shortening the time for equilibration and improving data quality.

For multi-angle light scattering (MALS) applications, columns with low noise levels yield high signal-to-noise ratios and this will improve sensitivity of detection. For mass spectrometry applications, low shedding columns will increase electrospray ionization efficiency and enhance overall MS performance and instrument uptime.

Figure 1. Calibration Curve of TSKgel UP-SW3000-LS

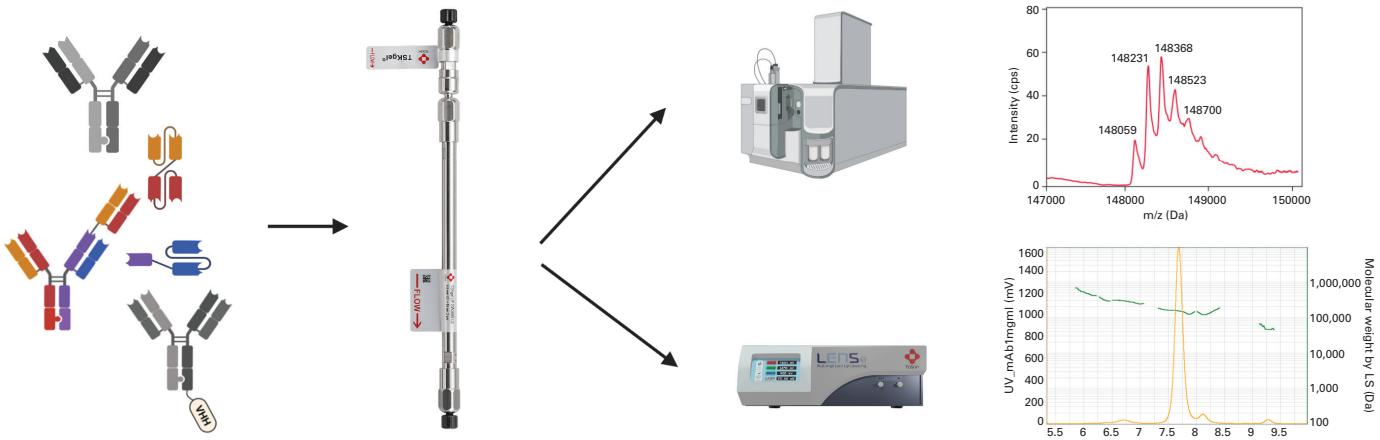


Column:	TSKgel UP-SW3000-LS 4.6 mm ID × 30 cm
Mobile phase:	100 mmol/L phosphate buffer pH 6.7 + 100 mmol/L, Na_2SO_4
Flow rate:	0.35 mL/min
Sample:	1 & 1' - thyroglobulin monomer (640 kDa) and dimer (1280 kDa) 2 & 2' - gamma-globulin (155 kDa) and dimer (310 kDa) 3 - ovalbumin (47 kDa) 4 - ribonuclease A (13.7 kDa) 5 - p-aminobenzoic acid (137 Da)

Short profile of TSKgel UP-SW3000-LS

Separation mechanism:	size exclusion
Base material:	diol-bonded silica
Particle size:	2 µm
Pore size:	25 nm
Hardware:	stainless steel
Dimensions:	4.6 mm ID × 30 cm L and 4.6 mm ID × 15 cm L, guard columns available
Separation range (proteins):	10 kDa – 500 kDa (see *Figure 1*)
Applications:	MALS and MS analysis of biotherapeutics (see *Figure 2*)

Figure 2. SEC Applications of the TSKgel UP-SW3000-LS Columns with Advanced Detection



mAbs, bispecifics, multispecifics, modern biotherapeutics

TSKgel UP-SW3000-LS for advanced detection methods

Characterize molecular weight, size, isoforms using MALS or mass spectrometry

Illustrations created with BioRender.com

Advanced columns for advanced detection

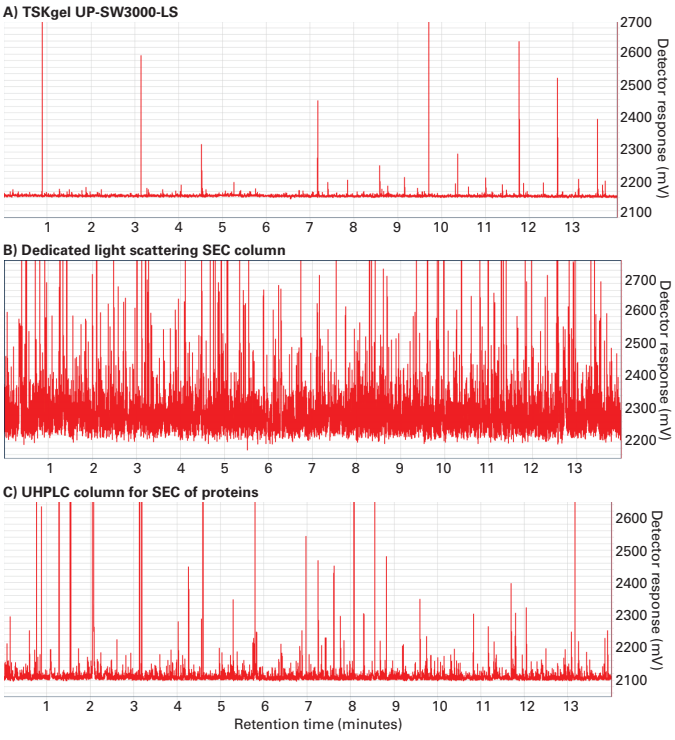
Recently, more focus has been placed on biomolecules derived from monoclonal antibodies, such as bi- or multi-specifics. These are potentially more complex molecules with increasing low and high molecular weight impurities. An advanced detection method such as MALS or mass spectrometry is needed to characterize these complex samples. The TSKgel UP-SW3000-LS column is designed to be coupled with an advanced detection instrument to achieve these analyses.

How the TSKgel UP-SW3000-LS performs *Low noise in MALS and MS applications*

The main feature of the TSKgel UP-SW3000-LS column is its reduced noise, as is demonstrated in *Figure 3* (panel A). Data of the low angle signal acquired from the LenS3 multi-angle light scattering detector shows very low shedding levels from the column.

For comparison, the mobile phase was injected to three different columns, the TSKgel UP-SW3000-LS column (*Figure 3*, panel A), a competitive light-scattering dedicated column (*Figure 3*, panel B), and a non-Tosoh UHPLC column intended for biopharmaceutical analysis (*Figure 3*, panel C). These results clearly show that the TSKgel UP-SW3000-LS column provides the lowest amount of spikes and the lowest noise level.

Figure 3. Comparison of HPLC and UHPLC Columns for MALS

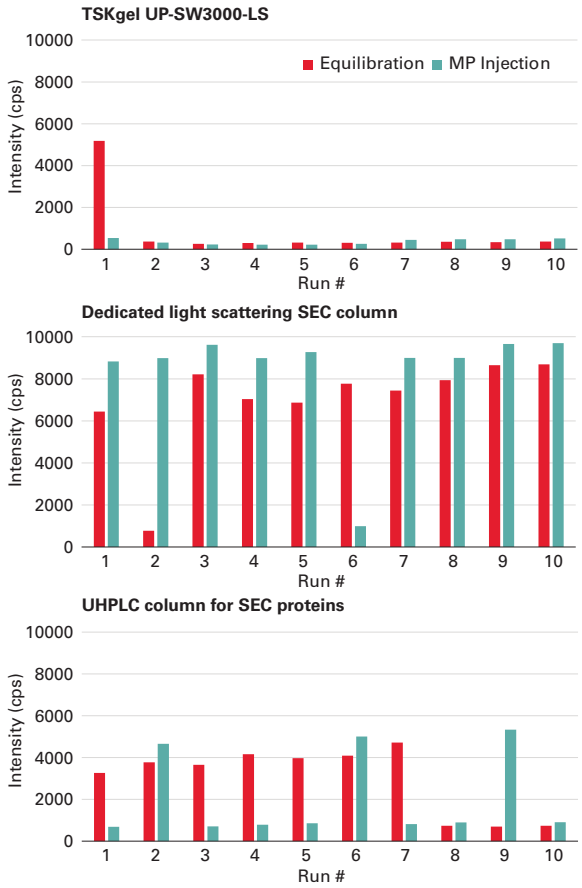


Comparison of HPLC and UHPLC columns for multi-angle light scattering applications. Low angle scattering signal (LALS) was detected by the LenS3 multi-angle light scattering detector. For each column, the third injection is shown.

Columns:	all 4.6 mm ID × 30 cm L
Mobile phase:	100 mmol/L phosphate buffer pH 6.7 + 100 mmol/L, sodium sulfate
Flow rate:	0.35 mL/min
Run time:	15 min
Injection volume:	10 µL

The reduction of shedding in mass spectrometry applications is demonstrated in *Figure 4*. The cumulated intensity during equilibration and after injections of the mobile phase which represents the presence of column shedding, is shown. The TSKgel UP-SW3000-LS delivers the lowest "shedding" signals, which is achieved during one equilibration cycle of 20 min.

Figure 4. Comparison of HPLC and UHPLC Columns for ESI-MS



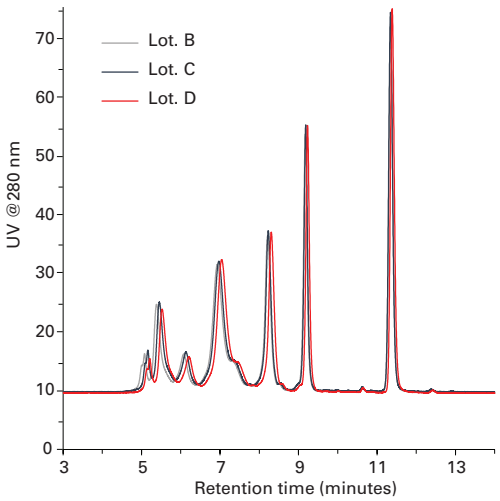
Comparison of the ions/shedding visible on three different columns in ESI-MS detection. Shown is the cumulated intensity over 12 minutes for equilibration (without injection) and for the time after injection with the mobile phase.

Columns:	all 4.6 mm ID × 30 cm L
Mobile phase:	100 mmol/L ammonium formate, pH 6.8
Flow rate:	0.2 mL/min
Injection volume:	10 µL

Lot-to-lot reproducibility and lifetime

In addition to MALS and MS detection compatibility, the TSKgel UP-SW3000-LS column provides high lot-to-lot reproducibility for reliable analyses as shown in *Figure 5*. This is further supported by a lifetime of > 500 injections (tested with intermittent stop of the flow) as demonstrated in *Table 1*. These characteristics make the column an efficient and reliable partner to analyze demanding biotherapeutic molecules.

Figure 5. Lot-to-Lot Reproducibility of TSKgel UP-SW3000-LS



Separation of a protein standard on three columns of different lots.

Column:	TSKgel UP-SW3000-LS 4.6 mm ID × 30 cm L
Mobile phase:	100 mmol/L phosphate buffer pH 6.7 + 100 mmol/L, sodium sulfate
Flow rate:	0.35 mL/min
Sample:	Protein standard containing thyroglobulin (640 kDa) gamma-globulin (155 kDa) ovalbumin (47 kDa) ribonuclease A (13.7 kDa) p-aminobenzoic acid (137 Da)

Table 1. Column Lifetime

Inj.	Elution time (min)	Elution time variation (%)	Theoretical plates (TP)	TP variation (%)
1	12.464	100.0	55748	100.0
101	12.448	99.9	56343	101.1
201	12.455	99.9	56064	100.6
301	12.460	100.0	56055	100.6
401	12.443	99.8	55792	100.1
500	12.443	99.8	55792	100.1

An antibody was repeatedly injected onto a single TSKgel UP-SW3000-LS column, after 100 injections flow was stopped for 6 h before continuation of antibody injections. p-Aminobenzoic acid was injected to test performance (elution time and theoretical plates).

Ordering Information

Part #	Description	Dimension (ID x cm)	Matrix	Pore size	Housing
0023546	TSKgel UP-SW3000-LS, 2 µm	4.6 mm ID × 30 cm L	Silica	25 nm	Stainless steel
0023547	TSKgel UP-SW3000-LS, 2 µm	4.6 mm ID × 15 cm L	Silica	25 nm	Stainless steel
0023548	TSKgel guard column UP-SW-LS	4.6 mm ID × 2 cm L	Silica	25 nm	Stainless steel
0023549	TSKgel guard column UP-SW-LS DC*	4.6 mm ID × 2 cm L	Silica	25 nm	Stainless steel

** DC: direct connect*