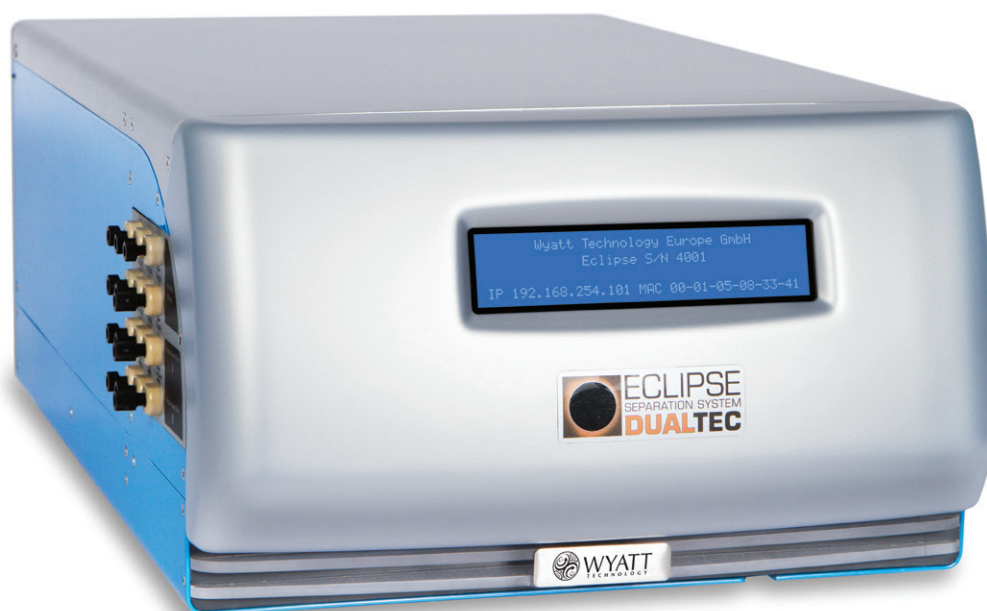


Eclipse

DualTec, AF4 & Mobility

Premier Flow-FFF technology for versatile separations



Eclipse®

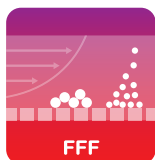
Separate and characterize proteins, polymers and colloids

Separate & Conquer

Asymmetric-Flow Field-Flow Fractionation (AF4) is an advanced technique for achieving analytical and semi-preparative separations, applicable to a wide range of analytes including:

- Proteins
- Polymers
- Nanoparticles
- Colloids
- Complex fluids

Covering an extraordinary range of sizes from 1 nm to 10 µm, AF4 offers programmable separation power yet negligible shear. It does not disrupt delicate macromolecular assemblies or loose protein aggregates.



Even the most complex macromolecular solutions and colloidal particle suspensions can be analyzed utilizing multiple online detection methods after separation by AF4, from light scattering to ICP-MS.

Hollow-Fiber Flow Field Flow Fractionation (HF5), closely related to AF4, performs low-volume analytical separations in convenient disposable cartridges with nanograms of analyte.

Meet the Family

The Eclipse family of flow field-flow fractionation controllers and separation channels offers multiple options to meet the most demanding separation needs.

Eclipse DualTec The 5th generation AF4 controller incorporates novel tip injection technology to support HF5

Eclipse AF4 The 4th generation AF4 controller supports semi-preparative and frit-inlet channels

Analytical Separation Channels Two sizes to optimize separation power and sample quantity in the microgram range

Mobility Separation Channel A new hardware design allows for applying the electrical field directly inside the channel. The connection is made by magnetic contacts without any wires to be attached manually.

Mobility Control Unit Aside of the high precision power-supply to generate the electrical field inside the channel the control unit also contains a Eclipse® conductivity detector and a pH sensor. It constantly provides these data to the analysis software to be displayed in the user interface.

HF5 Cartridges Disposable channels for nanogram separations

Semi-preparative Channel Fractionate milligrams of sample

Frit-inlet Channel Suitable for aggregation-prone samples

	Eclipse DualTec	Eclipse AF4
Description	Most versatile separations	Specialized separations
Tip Injection	For HF5	No
Dual-channel Switching	Any two of SEC, AF4, HF5	Optional
Metal-free Flow Path	For ICP-MS	No
Temperature-controlled Separations	4 °C to 90 °C with a temperature regulation chamber	
Channel Options		
Analytical AF4	Long and Short Channels 1 to 100 µg injections	
Disposable Hollow Fiber	pg to low µg injections	No
Semi-preparative	No	mg separations
Frit-inlet	No	For aggregation-prone samples
Mobility	Zeta potential	Zeta potential

Building blocks for FFF-MALS

In FFF-MALS, the HPLC modules and Eclipse perform the separation tasks, but not analysis

The Eclipse separation system integrates with high-quality HPLC components, including pumps, autosamplers, detectors and fraction collectors, from some of the leading HPLC manufacturers: Agilent, Thermo (Dionex), and Shimadzu.

Eclipse control plug-ins have been created for the OpenLab®, ChemStation®, Chromeleon® and LabSolution® CDS, taking advantage of wide familiarity with those packages.

Wyatt can configure your current HPLC instruments for FFF-MALS, or provide a complete turn-key solution with your choice of HPLC from these vendors.

The Eclipse Mobility™ is the most versatile solution for electrical asymmetric flow field flow fractionation (EAF4) which combines innovative channel design with outstanding software control and analysis. Being selective according to size and particle charge it optimizes the resolution of species which differ in charge, but cannot be separated on size alone. In addition the Eclipse Mobility simultaneously determines the electrophoretic mobility even of multimodal and polydisperse populations.

Analysis of the analyte's molecular weight, size and conformation falls to the detectors:

- DAWN® HELEOS® II, the essential MALS detector for characterizing molar mass from 200 Da to 1 GDa and size (rms radius R_g) from 10 nm to 500 nm
- WyattQELS™ dynamic light scattering module, embeds inside the DAWN, for measuring size (hydrodynamic radius, R_h) from 0.5 to 300 nm
- Optilab® T-rEX,™ the leading differential refractometer for universal concentration measurement
- ViscoStar III,® the latest technology in differential viscometers for characterizing polymer conformation and size



Eclipse DualTec

More automation and flexibility

Automated switching

Afraid that your HPLC system will be tied up with FFF? SEC may be your primary separation technique, and FFF only used as an orthogonal method.

The DualTec gives you the best of both worlds: it switches automatically between any two of SEC, AF4 and HF5, using the same HPLC and detector modules for each method.

- Compare results on SEC and HF5
- Develop a method on HF5 and transfer to AF4
- Test AF4 channels with different lengths and spacer thicknesses

The DualTec's automated switching provides the versatility to run proteins in the morning on SEC or HF5, and nanoparticles on AF4 in the afternoon—even making the switch during an all-night sequence.

Automated focusing

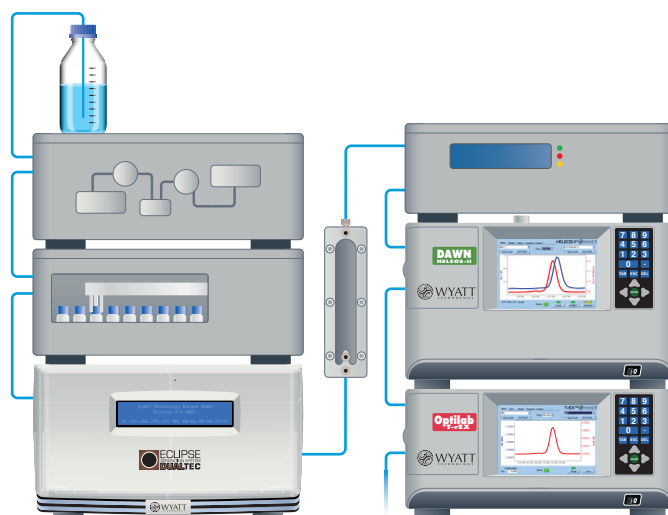
In order to achieve optimal separation, the sample must be focused to a narrow band near the channel inlet prior to elution. In other flow-FFF systems, the focusing operation is performed manually by the operator using a colored sample to observe the band. The DualTec's unique AutoFocus feature relieves the operator of this task, making for a seamless workflow from injection to analysis. It also removes the requirement for a transparent upper plate in the separation channel.

Metal-free option

The metal-free flow path option switches to a completely metal-free path at the push of a button. This configuration is optimal for using the Eclipse with ICP-MS, where metal ions can interfere with the analysis.

Additional benefits of the DualTec

- Tip-injection technology requires only inlet and outlet ports for the separation channel, greatly simplifying the system relative to the traditional three-port configuration. Tip injection facilitates automated switching and HF5 cartridge use.
- Dual pressure transducers monitor the pressure drop across the membrane to alert users for health and clogs.
- Advanced Coriolis-force mass flow sensors are highly accurate and require no calibration or compensation for solvent type, pressure or temperature.

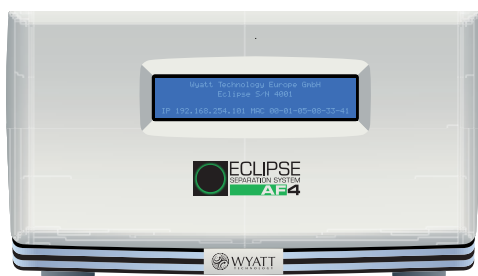


Eclipse AF4

Frit inlet and semi-preparative capabilities

The AF4 Standard

Users of the Eclipse AF4 system enjoy the benefits of the essential Eclipse approach to flow-FFF: the unique single-pump design, which does away with the complication, reduced reliability and increased pulsation of a multiple-pump flow-FFF system.



The Eclipse AF4 retains the conventional three-port flow-FFF design, and does not support tip-injection or HF5. However, for specific applications it offers specialized separation modes:

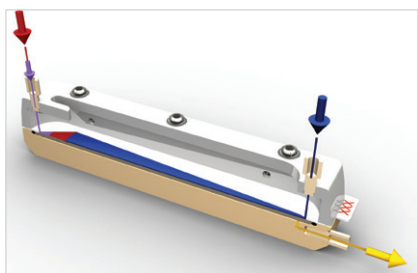
- Frit-inlet channel: some samples, such as proteins or high-molecular-weight polymers, may be prone to aggregation during the focusing step which concentrates the sample on the membrane. The AF4 supports Wyatt's unique FI (frit inlet) channel that eliminates the need to focus the sample, and prevents aggregation.
- Semi-preparative: The AF4's high-capacity sample injection fluidics can load milligrams of protein, up to 100x more than a typical analytical AF4 injection. Wyatt's unique SP (semi-preparative) channel can be used with the HPLC's fraction collector to pool purified fractions for later use.

How Asymmetric-Flow FFF works...

Gentle and fast one-phase separation

In AF4, there is no stationary phase; the channel constitutes an open flow path between two parallel plates. The top plate is solid and the bottom plate is a semi-permeable membrane supported by a frit. While solvent can pass through the membrane, sample larger than the membrane cutoff size cannot.

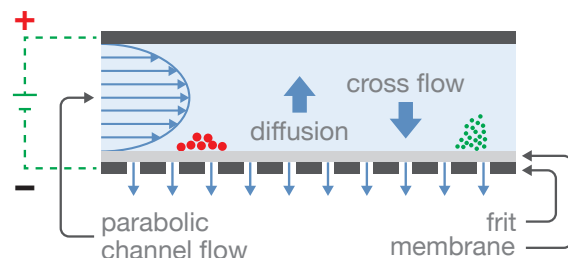
A flow restrictor on the channel outlet forces a portion of the flow, known as the cross flow, to pass through the membrane. Samples are introduced into the flow stream at the top of the separation channel (red arrow below) and focused along a narrow band of the membrane by opposing channel flows (red and blue arrows) which pass through the semi-permeable membrane.



The DualTec AF4 channel uses tip-injection. The sample is introduced with the flow entering the channel inlet prior to focusing.

The cross-flow force pushing the sample against the membrane is balanced by diffusion, resulting in an equilibrium height distribution relative to the membrane that depends on particle size. Smaller particles are, on average, higher up in the channel compared to larger particles.

Asymmetric Flow Field-Flow Fractionation (AF4) Flat Channel



FFF separation power can be tuned by changing the ratio of cross flow to channel flow. Additional charge-based separation is provided by the Mobility electrical AF4 channel.

After establishing equilibrium height distributions, channel flow is applied parallel to the membrane, creating a laminar flow stream with a parabolic velocity profile. The flow velocity strongly increases with its distance from the wall. Hence smaller particles are transported more rapidly along the channel relative to larger particles. Finally, the particles are swept out of the channel, downstream to the detectors, eluting in order of increasing size.

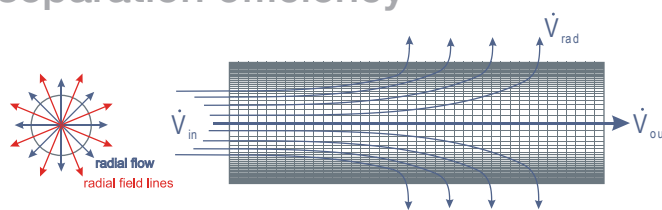
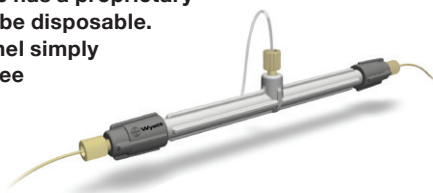
...and Hollow-Fiber Flow-FFF

Higher sensitivity and separation efficiency

Hollow-Fiber Flow-FFF (HF5) uses a tubular polymeric or ceramic fiber with porous walls as the separation channel. Cross flow in the fiber is radial instead of unidirectional. These fibers allow a high flux, are mechanically stable, and exhibit minimal interaction with most types of samples, particularly proteins.

HF5 offers three primary advantages over AF4: disposable cartridges, higher sensitivity, and higher separation efficiency (plate numbers). In HF5, the same retention results in 33% lower concentration levels compared to AF4. This helps to prevent overloading and sample aggregation or absorption. HF5 encapsulates 10x lower volume compared to a typical AF4 channel, with correspondingly narrower peaks.

The HF5 channel cartridge has a proprietary design and is intended to be disposable. Replacement of the channel simply requires reconnecting three finger-tight nuts.



HF5 presents a simple geometry: the cross flow is radially symmetric. The separation channel consists of a semi-porous, hollow tube, in one continuous piece.

Detector flow rates can also be reduced, leading to lower dilution factors. Together, narrow peaks and low dilution contribute to increased sensitivity. A side benefit of these is greatly reduced solvent consumption.

The disposable HF5 channel is a major break-through in FFF technology, as it greatly simplifies the effort of membrane replacement. Still, an HF5 cartridge will typically be usable over 100 injections.

The combination of long life, convenient and inexpensive replacement, reduction in sample and solvent consumption, and faster run times adds up to a major productivity boost to FFF users.

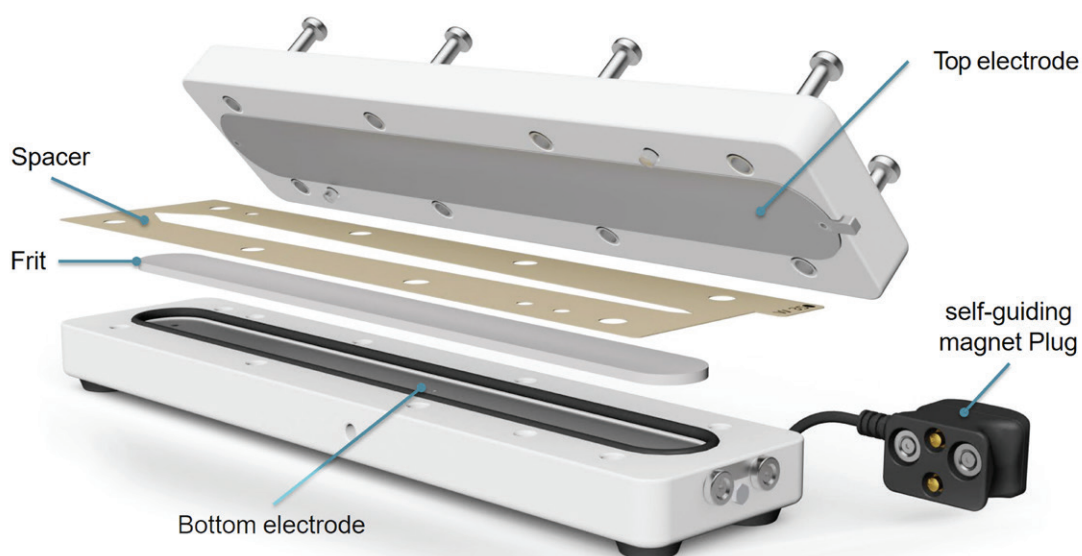
Mobility

Electrical charge in FFF

The Eclipse Mobility is the most versatile solution for electrical asymmetric flow field flow fractionation (EAF4) which combines innovative channel design with outstanding software control and analysis. Being selective according to size and particle charge it optimizes the resolution of species which differ in charge, but cannot be separated on size alone. In addition the Eclipse Mobility simultaneously determines the electrophoretic mobility even of multimodal and polydisperse populations. Hereby a series of measurements is performed automatically at different field strengths to receive the highest possible data quality.

Mobility Control Unit

Aside of the high precision power-supply to generate the electrical field inside the channel the control unit also contains a Eclipse conductivity detector and a pH sensor. It constantly provides these data to the analysis software to be displayed in the user interface.



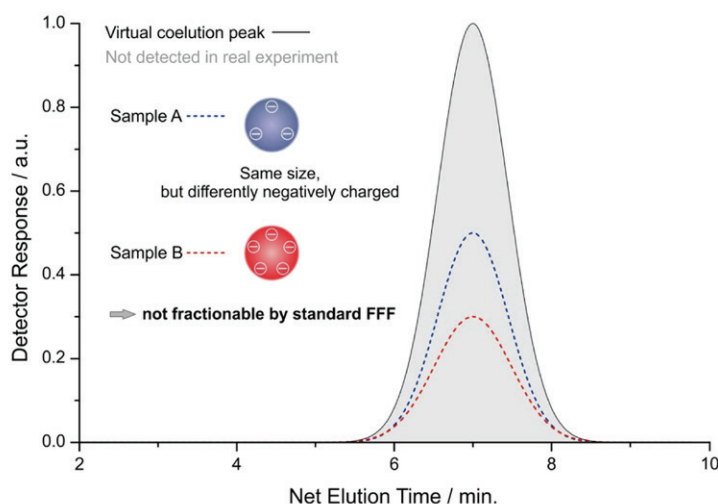
Mobility Separation Channel

A new hardware design allows for applying the electrical field directly inside the channel. The connection is made by magnetic contacts without any wires to be attached manually.

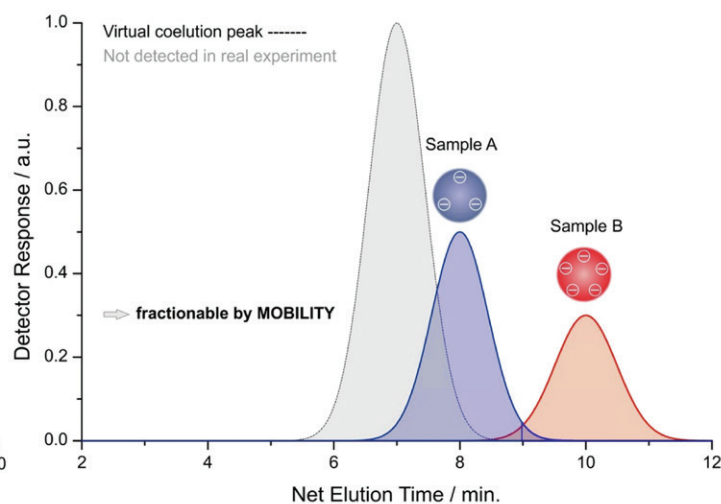
What do I get from applying an electric field to an FFF channel?

- ✓ Improved separation performance for standard FFF
- ✓ Electrophoretic mobility measurements for fractions or distributions

Without E-field



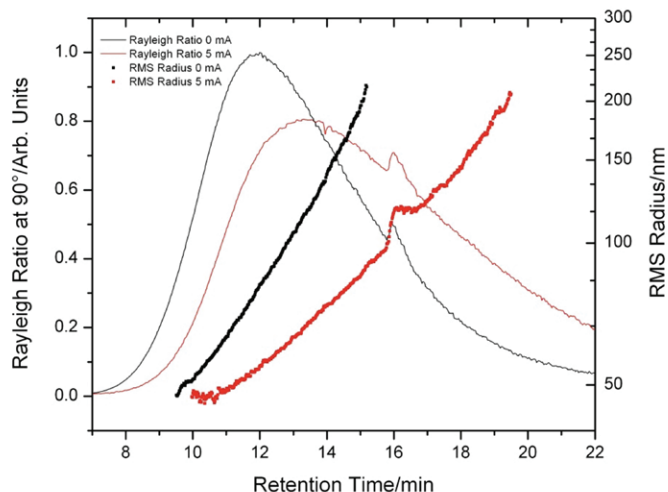
With E-field



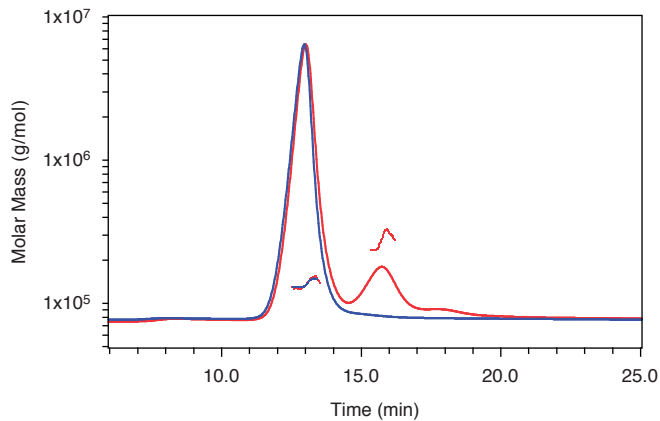
Lipoplex size and charge characterization

EAF4 experiment on a negatively charged lipoplex sample without (black solid line) and with (red solid line) applied electric field together with the corresponding rms radius distributions, ranging from ≈ 50 nm to 200 nm, with the bottom electrode being the anode and thus positively charged. The measurement with applied electric field shows a clear shift towards higher retention times and an increased resolution of the rms radius distribution, which confirms the negative charge of the lipoplex qualitatively. Since the electric field acts just like an additional cross-flow field, the electrophoretic mobility can also be determined quantitatively, as shown for some selected rms radii. All values are above $-6 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, indicating a stable dispersion.

R_g/nm	$\mu/\text{m}^2 \text{ V}^{-1} \text{ s}^{-1}$	ζ/V
52	-6.52×10^{-8}	-0.0919
60	-6.85×10^{-8}	-0.0955
85	-7.75×10^{-8}	-0.1063
95	-7.75×10^{-8}	-0.1059
120	-7.34×10^{-8}	-0.0995
135	-7.39×10^{-8}	-0.0999

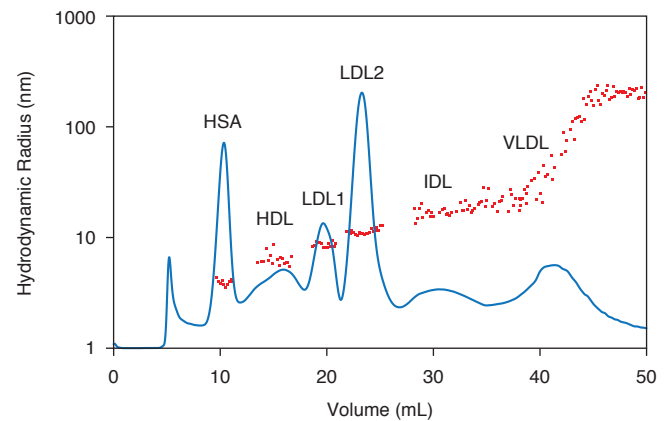


Monoclonal antibodies and aggregates



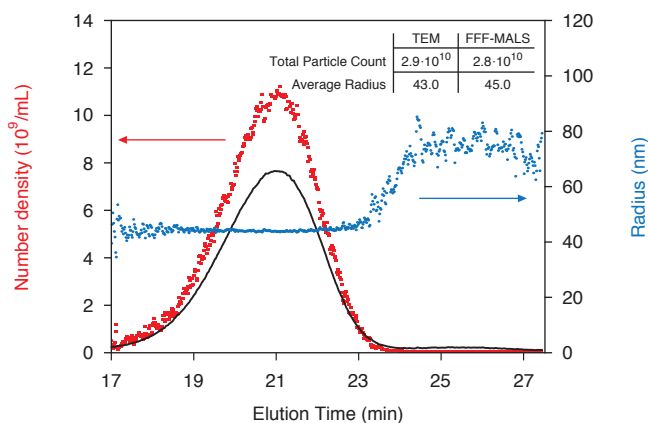
HF5-MALS analysis of two different preparations of a monoclonal antibody (mAb), using an Eclipse Dualtec and an HF5 cartridge: I (UV trace in blue) and II (red). HF5 exhibits excellent resolution between monomer, dimer and higher oligomers, with high reproducibility. The injected mass was only 2 ug.

Whole serum



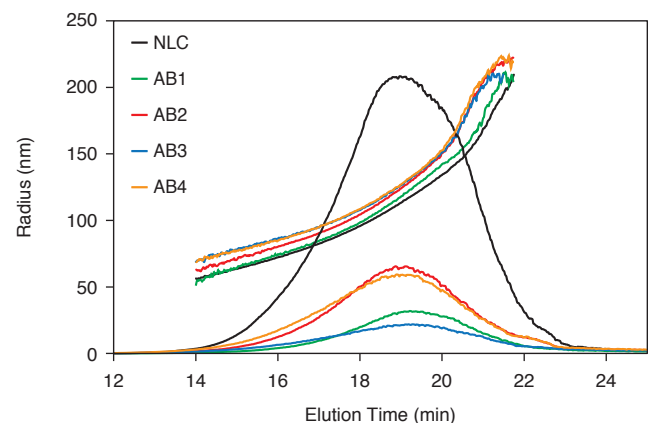
AF4-MALS-DLS separation of whole serum, with distinct peaks for serum albumin, IgG, and various types of lipoproteins. Sizes (R_h) were determined by online dynamic light scattering embedded in the DAWN MALS detector. Not shown, MALS also determines molar masses of each peak and, for species larger than ~ 10 nm, rms radius R_g .

Adenovirus concentration



AF4-MALS provides quantitative, high-resolution size distributions with large particle ensembles for statistical certainty. This adenovirus analysis indicates the number density in billion/mL at each elution time, along with the radius, both of which compare very well with TEM. The LS fractogram is overlaid in black. A small fraction of dimers is evident.

Silver-nanolipid complexes



AF4-MALS analysis of a nanolipid complex (NLC) and microsilver-loaded NLC formulations with varying concentrations of NLC and microsilver. All preps show a higher radius than the pure NLC, proving the adsorption of silver ions. The size saturated at 10% w/w NLC and 0.1% w/w Ag. Reproducibility is better than 1%.

Specifications

Separation Range	15 ng of 100 kDa polystyrene in THF or 300 ng of BSA in PBS, assuming standard 7.8 mm x 300 mm SEC column
Control Unit	
Solvent Compatibility	<i>Standard Version:</i> For aqueous solvents and alcohols. Corrosion resistant from pH 2 to 12. Wetted materials: PEEK, stainless steel, Kalrez; <i>Organic Version:</i> For organic solvents such as THF, toluene, etc. Includes vapor sensor with interlock output. Wetted materials: PEEK, stainless steel, Kalrez; <i>Metal-Free Version (DualTec only):</i> Wetted materials: PEEK
Supported HPLC Software Control	Agilent, Thermo, Shimadzu Eclipse software plug-ins for Agilent OpenLab and Chemstation, Thermo Chromeleon and Shimadzu LabSolution; VOYAGER CDS® stand-alone chromatography data system controls Eclipse, Agilent HPLC modules and ASTRA software to initiate detector data acquisition
Additional Software	SCOUT DPS® for virtual method development, method optimization and particle size analysis
Communications Inputs/Outputs	Ethernet Autoinject In, Autoinject Out, Analog In, Pump Analog Control Out, Detector Zero Out, Start Pump, Pump Interlock for Agilent pumps
Dimensions	64 cm (L) x 36 cm (W) x 25 cm (H)
AF4 Channels Selection	Short Channel, 153 mm e.l.; Long Channel, 246 mm e.l.. Eclipse AF4 supports the Semi-Prep Channel, 246 mm e.l. and Frit-inlet Channel 246 mm e.l. Each channel is available in aqueous and organic version
Mobility Wetted Materials	Electrical field based channel and electrophoretic mobility module Aqueous channels: Stainless steel, PEEK, polycarbonate; Viton Organic channels: Stainless steel, PEEK, Kalrez
Channel Pressure	Maximum 30 bar. Software incorporates safety shut-down for overpressure
Spacers Membranes	Spacer thicknesses are 190, 250, 350 and 490 µm Pre-cut: Polyether Sulfone: 1, 3, 5, 10, and 30 kDa cut off; Cellulose Triacetate: 10 kDa cut off; Regenerated cellulose: 5, 10, and 30 kDa cut off (availability subject to change without notice). Other membranes may be cut from sheets with the Membrane Template Kit.
Hollow-Fiber Cartridge Selection	PES membrane 10 kDa cutoff, channel volume 90 µl, e.l. 170 mm, channel height 400 µm. Aqueous solvents only.

* Depending on dn/dc , the sample concentration and chromatography conditions, this is typical.

† Configuration and flow-rate dependent.

Wyatt Technology is committed to continual improvement. Specifications subject to change without notice.

Warranty: All Wyatt instruments are guaranteed against manufacturing defects for 1 year.

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Left to Right

Geoffrey K. Wyatt, Chief Executive Officer
Dr. Philip J. Wyatt, Chairman of the Board
Clifford D. Wyatt, President

For more than 35 years, we've operated as one of the very few remaining family-owned businesses in the analytical instrument industry. With installations in more than 65 countries, over 14,000 refereed journal publications citing our instruments and more than 25 PhD scientists on staff, we take great pride in the worldwide recognition that Wyatt Technology has received as a leading manufacturer of instruments and software for absolute macromolecular and nanoparticle characterization. Our dedication to providing customers with comprehensive training and personal support has made us the gold standard in this field.

The Eclipse is one of many tools in Wyatt's Light Scattering Toolkit for Essential Macromolecular and Nanoparticle Characterization.

Learn more at www.wyatt.com