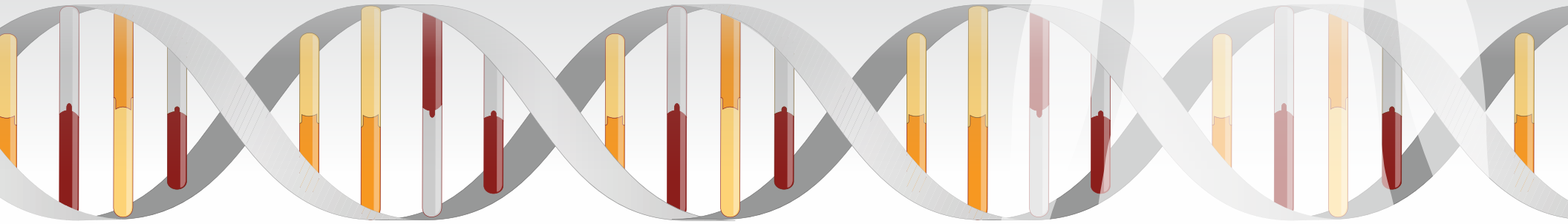
ThermoFisher SCIENTIFIC

Gene therapy analytical solutions

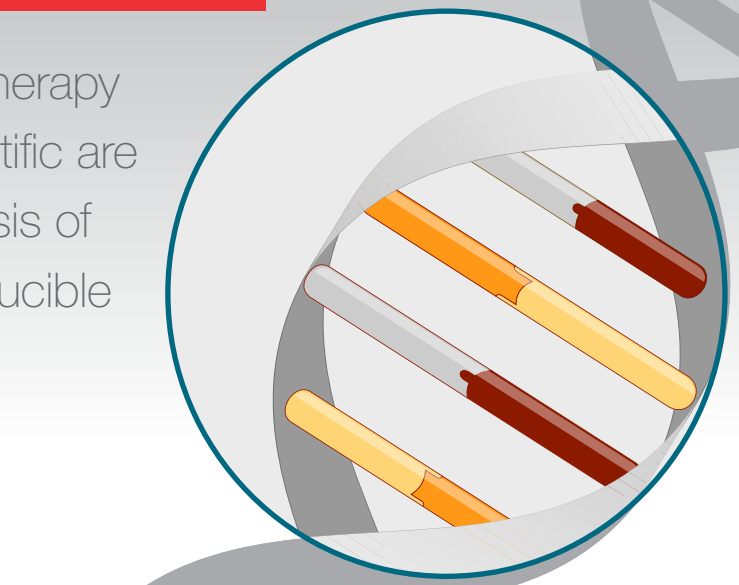
Fast, accurate, and scalable methods for gene-therapy-based product manufacturing, analytical testing, and characterization



thermo scientific

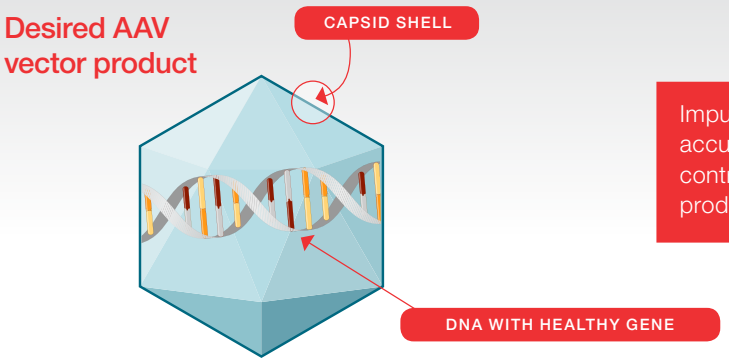
Fast, robust, and confident analysis from characterization through to control

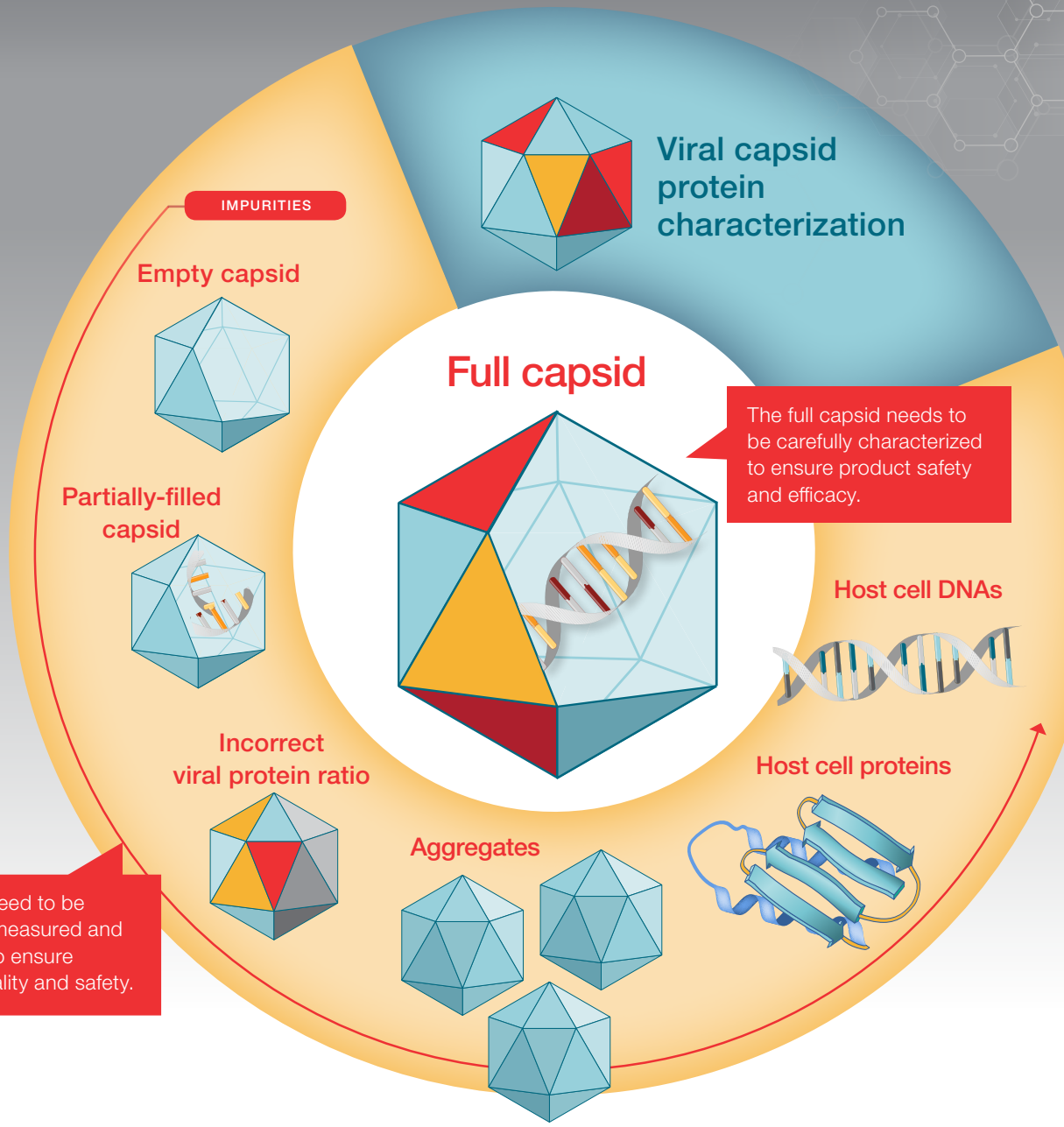
Technologies for manufacturing and the analysis of gene therapy products are evolving. Solutions from Thermo Fisher Scientific are evolving too. Our robust solutions are tailored for the analysis of gene therapy products enabling fast, accurate, and reproducible results accelerating product development path to market.



The complexity of AAV gene therapy products

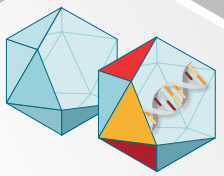
Adeno-associated virus (AAV) based gene therapeutics are far more complex than many traditional biotherapeutics. Besides the full capsid containing the desired gene material, the final product could contain many different types of process and product related impurities.



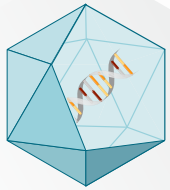


Critical quality attributes

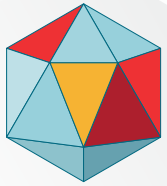
With the right analytical tools scientists can expedite development of safe, high-quality gene therapy products while reducing time-to-market and costs of manufacturing.



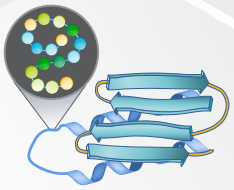
Empty/full capsid analysis



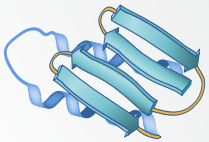
Intact mass analysis



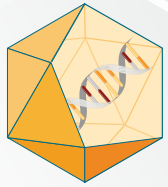
Capsid protein characterization



Capsid sequence and PTM analysis



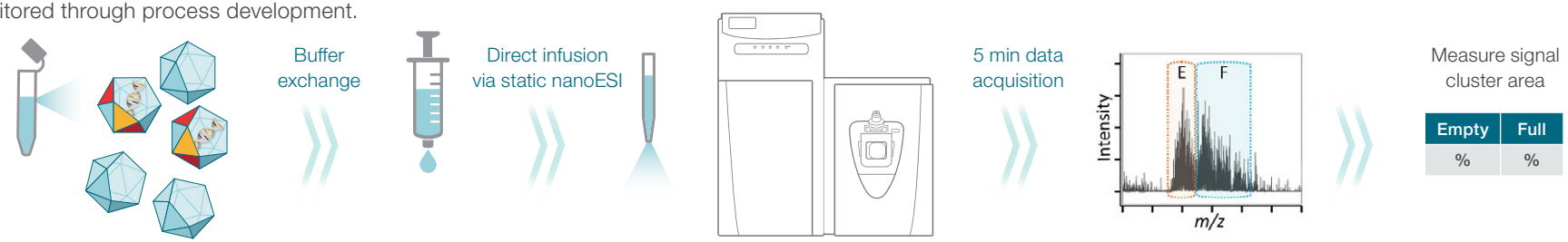
Host cell protein analysis

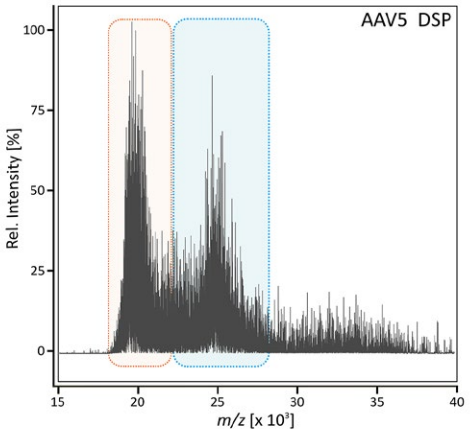


Titer

Native mass spectrometry-based approach to assess the ratio of empty to full AAV-capsids

- Empty capsids, which do not contain the gene therapy of interest, and partial capsids, those containing only a fragment of the gene of interest, are by-products of the AAV production and can impact product safety and efficacy.
- The amount of full, partial, and empty capsids, therefore, needs to be characterized and monitored through process development.
- A native MS-based approach provides the necessary improvements in sensitivity and selectivity when compared to UV and fluorescence based separation approaches, respectively.

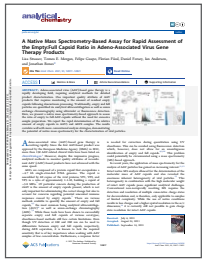




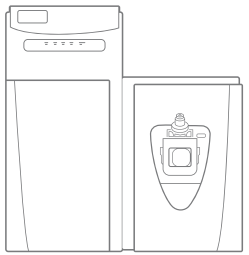
MS using the Q Exactive UHMR under native conditions can be used to reliably assess the empty to full ratio of AAV samples

- Full and empty capsids appear in different *m/z* regions
- Observed mass difference correlates with the mass of the incorporated cargo genome
- Relative quantification of the amount of full and empty capsids was provided
- Applicable for different serotypes

** Anal. Chem. 2021, 93, 38, 12817-12821*



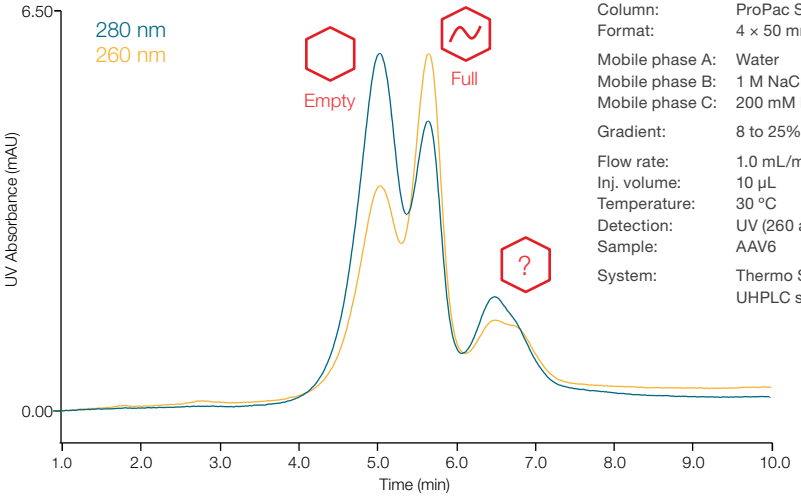
View complete article*



Thermo Scientific™ Q Exactive™ UHMR (Ultra-High Mass Range) Hybrid Quadrupole-Orbitrap™ Mass Spectrometer

Empty/full capsid analysis with anion exchange chromatography using ultraviolet detection

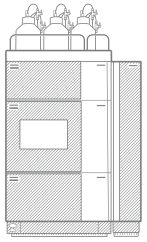
- AEX separation with UV detection provides selectively between empty and full capsids.
- Full capsids are highlighted with the increase in absorption at 260 nm corresponding to its nucleic acid content.
- The fast and robust empty/full capsid separation can be achieved using the Vanquish UHPLC system with ProPac SAX-10 column.
- The biocompatibility of the Vanquish Flex UHPLC system allows for the use of ion exchange and ion-pairing modifying buffer.



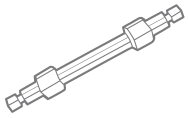
Column:	ProPac SAX-10
Format:	4 × 50 mm
Mobile phase A:	Water
Mobile phase B:	1 M NaCl
Mobile phase C:	200 mM Bis-tris propane, pH 9.5
Gradient:	8 to 25% B in 10 minutes; 10% C
Flow rate:	1.0 mL/min
Inj. volume:	10 µL
Temperature:	30 °C
Detection:	UV (260 and 280 nm)
Sample:	AAV6
System:	Thermo Scientific™ Vanquish™ Flex UHPLC system

The grafted anion-exchange surface provides pH-based selectivity control and fast mass transfer for high-efficiency separation

View webinar



Thermo Scientific™ Vanquish™ Flex UHPLC Systems



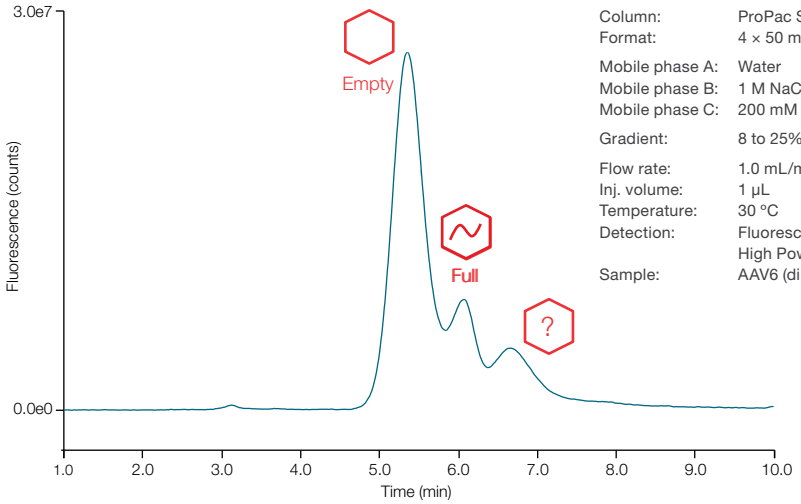
Thermo Scientific™ ProPac™ SAX-10 HPLC Columns

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software

Next page

Empty/full capsid analysis with anion exchange chromatography using fluorescence detection

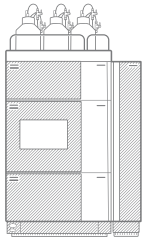
- AEX separation with fluorescence detection provides increases in sensitivity and accurate ratio information.
- The fast and robust empty/full capsid separation can be achieved using the Vanquish UHPLC system with ProPac SAX-10 column.
- The biocompatibility of the Vanquish Flex UHPLC system allows for the use of ion exchange and ion-pairing modifying buffer.



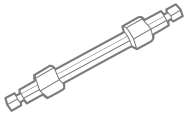
Column:	ProPac SAX-10
Format:	4 × 50 mm
Mobile phase A:	Water
Mobile phase B:	1 M NaCl
Mobile phase C:	200 mM Bis-tris propane, pH 9.5
Gradient:	8 to 25% B in 10 minutes; 10% C
Flow rate:	1.0 mL/min
Inj. volume:	1 µL
Temperature:	30 °C
Detection:	Fluorescence (Ex 280 nm / Em 330 nm) High Power, Sensitivity 8
Sample:	AAV6 (diluted 2-fold with DI water)

The grafted anion-exchange surface provides pH-based selectivity control and fast mass transfer for high-efficiency separation

View webinar



Thermo Scientific™ Vanquish™ Flex UHPLC Systems



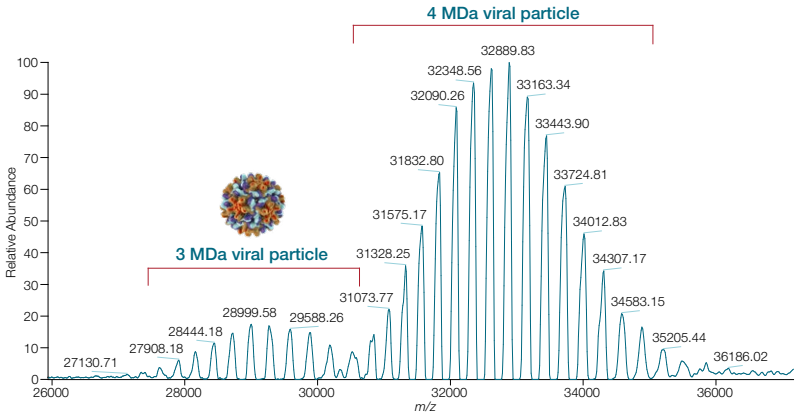
Thermo Scientific™ ProPac™ SAX-10 HPLC Columns

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software

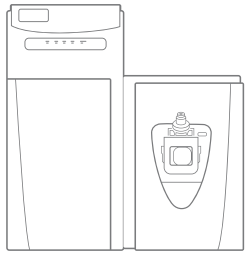
Understand heterogeneity at a new level with intact mass analysis

AAV capsid composition impacts viral infectivity and gene transduction. To ensure the safety and quality of gene therapies, AAV capsid and their constituent proteins need to be well characterized and monitored.

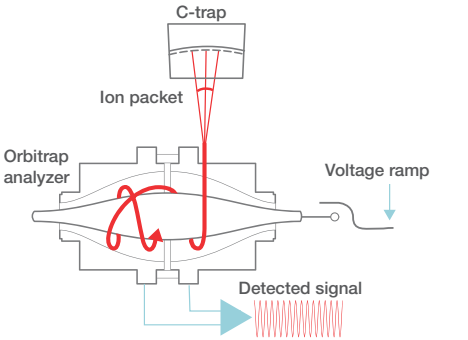
MS-based Intact mass analysis provides a robust and sensitive approach for capsid characterization. Mass confirmation can be performed with high confidence using the Q Exactive UHMR Hybrid Quadrupole-Orbitrap mass spectrometer. See how a mixture of 3 MDa and 4 MDa virus capsids analyzed under charge reducing conditions span a wide mass range between *m/z* 27,000 and 36,000.



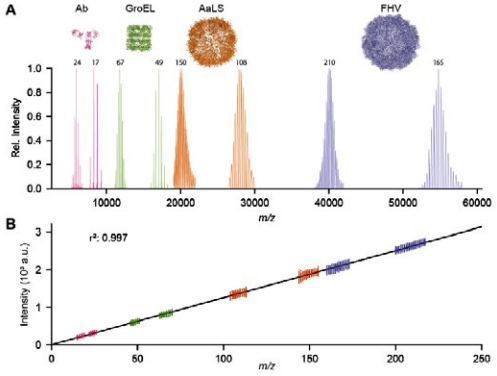
Viral vectors analysis with the Q Exactive UHMR hybrid quadrupole-orbitrap mass spectrometer



Thermo Scientific™ Q Exactive™ UHMR (Ultra-High Mass Range) Hybrid Quadrupole-Orbitrap™ Mass Spectrometer

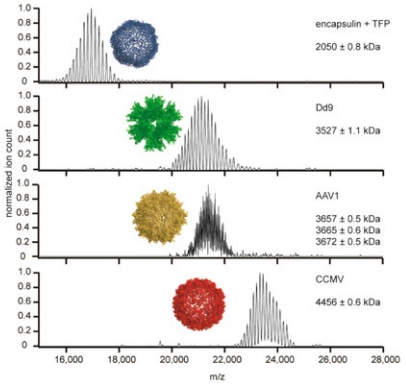


Schematic of the injection of ions from the C-trap into the Orbitrap, where they oscillate along the central electrode, inducing an image current, which is recorded and converted into a final *m/z* spectrum by Fourier transformation



Example native MS spectra

View webinar



Native MS of virus-like particles: Shown are encapsulin (top), adenovirus dodecahedron (topmiddle), adeno-associated virus serotype 1 (bottom-middle) and cowpea chlorotic mottle virus (bottom)

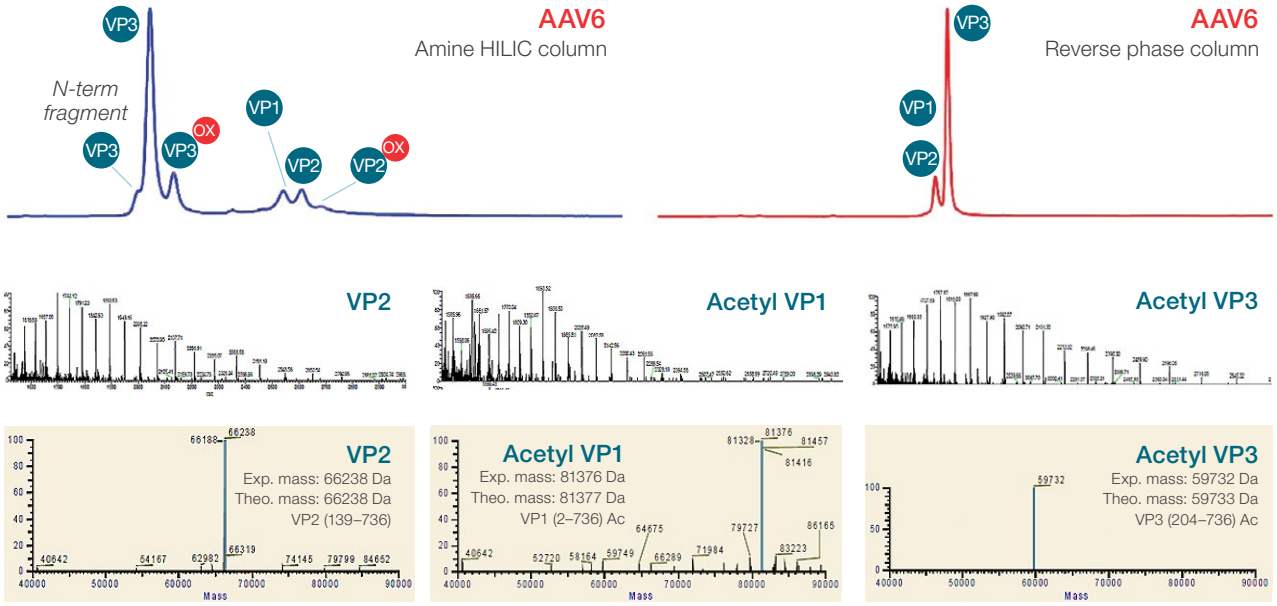
View webinar

Capsid protein characterization

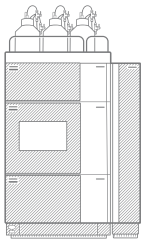
It is important to understand the capsid identity because each AAV serotype has unique capsid proteins that transfer the genetic material to specific cells or organs. Depending on the therapeutic target area, the correct AAV serotype is chosen and identified, then purity monitored. All AAV capsids consist of three proteins (VP1, VP2, and VP3) that share high-sequence homology. Ensuring the identity and purity of these proteins are critical to viral infectivity and viral transfer.

Identification of components

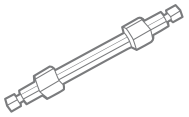
Elution order can change the serotype so positive identification by MS is an advantage



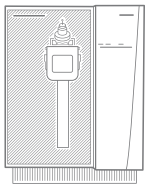
Separation of viral capsid proteins (VP1, VP2, VP3) via hydrophilic interaction liquid chromatography (HILIC, left) and reverse phase chromatography (right) with subsequent MS and MS/MS identification



Thermo Scientific™ Vanquish™ Flex UHPLC Systems



Thermo Scientific™ HILIC™ HPLC Columns

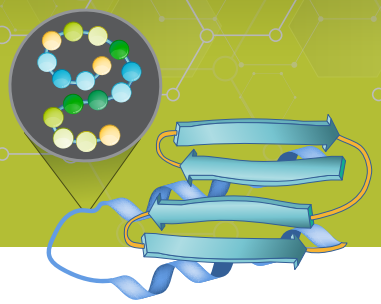


Thermo Scientific™ Orbitrap Exploris™ 480 Mass Spectrometer

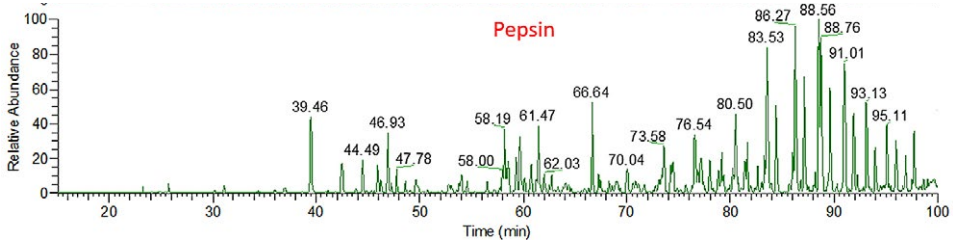
Thermo Scientific™ BioPharma Finder™ Software

Next page

Capsid protein characterization at the peptide level



The correct AAV serotype is selected, then identity and purity are monitored; either on the intact protein level or by peptide mapping. Peptide mapping can be performed using pepsin SMART digest kit, with state-of-the-art consumables and instrumentation, to increase viral protein 1 (VP1) coverage for confidence in your characterization and monitoring of viral capsids.



AAV2 100% coverage of VP1

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLDKGEPVNEADAAALEHD

KAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPD

SSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGN

Residues 182-245 GAP 1

WHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINN

NWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTL

Residues 361-389 GAP 2

NNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSR

LQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFF

PQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDRD

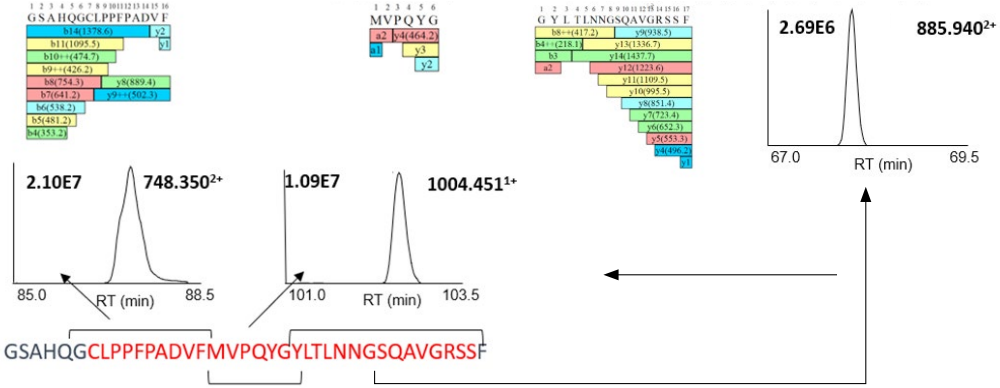
Residues 666-686 GAP 3

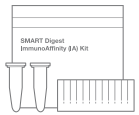
VYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKE

NSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

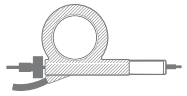
Trypsin
Pepsin

GAP 2

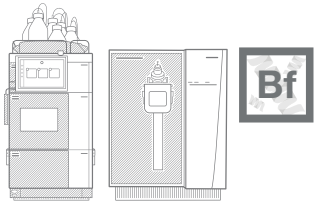




Thermo Scientific™ SMART Digest™ Pepsin Kit

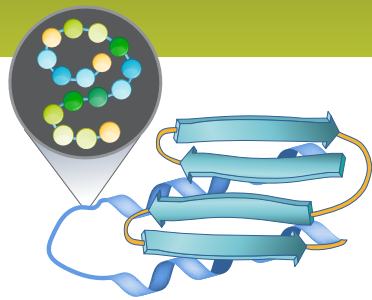


Thermo Scientific™ EASY-Spray™ PepMap Neo UHPLC column



Thermo Scientific™ Vanquish™ Neo UHPLC System, Orbitrap-based mass spectrometer and BioPharma Finder software

Capsid sequence and PTM analysis



Peptide mapping approaches

- Minimize sample handling
- Trap technology can help with clean up and concentration

Nanoflow for ultimate sensitivity

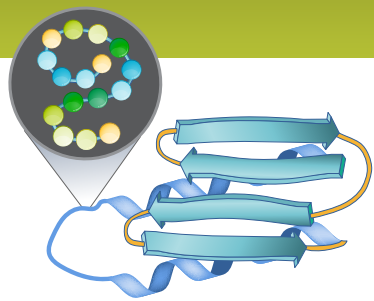
Thermo Scientific™ Vanquish™ Neo UHPLC Systems

1 or 2 mm columns for standard application

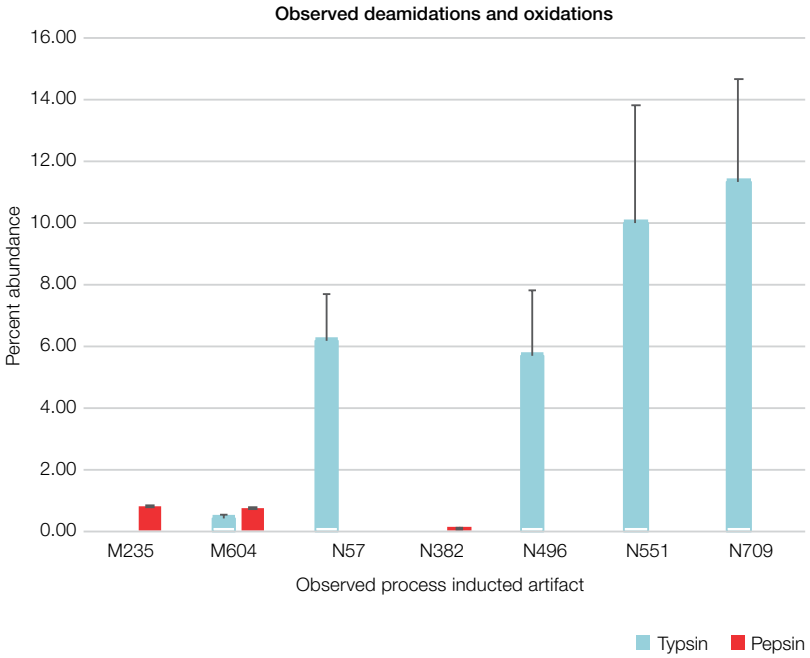
Thermo Scientific™ Vanquish™ Flex UHPLC Systems

Thermo Scientific™ Orbitrap Exploris™ 240 Mass Spectrometer

Capsid sequence and PTM analysis



Accurate monitoring of post translational modifications



Enzyme	M235	M604	N57*	N382*	N496*	B569*	N709*
Pepsin	0.80%	0.74%	0%	0.13%	0%	0%	0%
Trypsin	0%	0.43%	6.18%	0%	3.78%	6.64%	7.54%

- Trypsin digestion at pH 8 is known to create process induced deamidation
- Pepsin digestion at pH 2 does not create any process induced modifications
- Where the data is available for trypsin digestion the relative abundance of the PTM is in close agreement between trypsin and pepsin
- Pepsin produces more accurate data for monitoring PTM's in gene therapy capsids
- Less than 1% deamidation observed in peptic digest
- Less than 1% oxidation in all digests

Host cell protein analysis

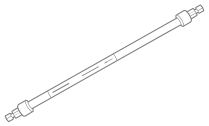
Host cell proteins (HCP) can impact quality or safety, or compromise product stability. Peptide analysis by LC-HRAM-MS offers orthogonal solutions for detection and monitoring of HCPs compared to immunological methods with the unbiased discovery of HCP impurities and subsequent quantitation. HRAM MS data combined with Thermo Scientific BioPharma Finder software provides comprehensive HCP qualitative and quantitative analysis.

LC-HRAM-MS Host cell protein analysis

1
Digestion

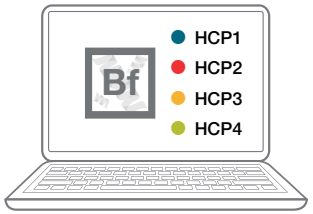
Peptide mixture

2
LC-MS/MS

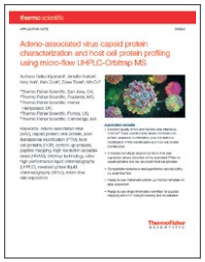


Acclaim VANQUISH C18 column

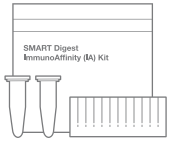
3
BioPharma Finder software



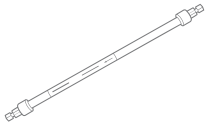
Host cell protein analysis



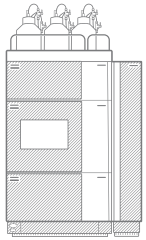
View complete application note



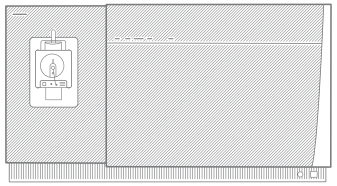
Thermo Scientific™ SMART Digest™ Trypsin Kits



Thermo Scientific™ Acclaim VANQUISH C18 UHPLC Columns



Thermo Scientific™ Vanquish™ Flex UHPLC Systems



Thermo Scientific™ Orbitrap Eclipse™ Tribrid Mass Spectrometer

Thermo Scientific™ BioPharma Finder™ Software

New products

Thermo Scientific™ EASY-Spray™ PepMap™ Neo UHPLC Columns

These columns feature easy connectivity, high reproducibilty, and excellent separations. Featuring low/zero dead volume for increased sensitivity in sample limited gene therapy applications.

Thermo Scientific™ Vanquish™ Neo UHPLC System

The Thermo Scientific™ Vanquish™ Neo UHPLC system delivers maximum performance 24/7 for nano-, capillary-, and micro-flow LC-MS. The system ensures excellent retention time precision from nano-flow up to 100 µL/min, the unique low-flow split-loop autosampler enhances sample throughput and injection performance. Vanquish Neo UHPLC system is the new standard in low-flow LC.

End-to-end solution provider

Unprecedented potential to treat diseases

Partnering with Thermo Fisher Scientific, with our expertise and unparalleled product portfolio, allows you to accelerate the development of your gene therapy-based products, by utilizing flexible, robust, fast, accurate, and scalable methods for product manufacturing, analytical testing, and characterization.

Plasmid construction

- Gene synthesis
- Cloning and assembly
- Large-scale plasmid purification

Vector production

- Gene delivery Virus-producing adherent and suspension cells
- Custom media current Good Manufacturing Practices (cGMP) chemicals and process liquids
- Specialized laboratory equipment

Vector purification

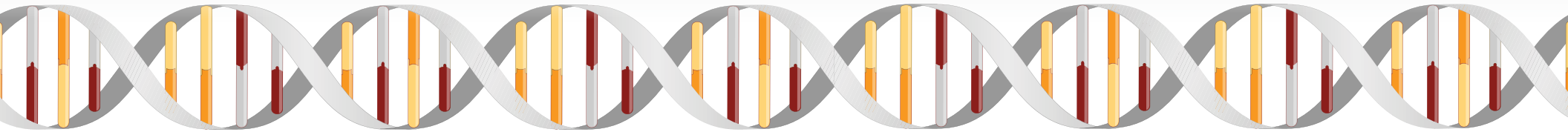
- Affinity chromatography
- Ion exchange chromatography
- Centrifugation
- Process liquid/buffer preparation services

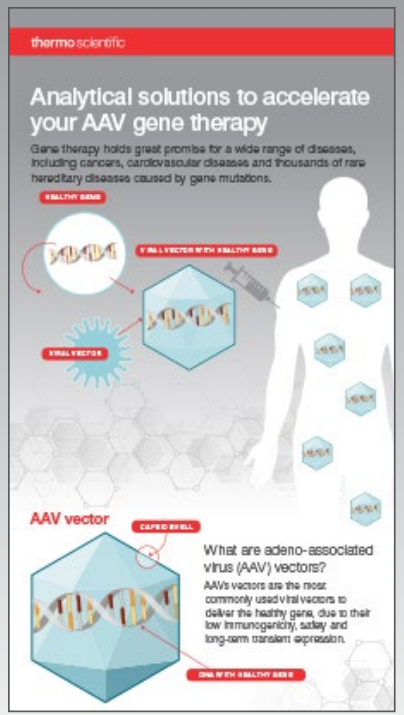
Lot release and characterization

- Identity, purity, and contamination
- Functional titer
- Empty capsid analysis
- Genomic, proteomic, and cellular analysis tools

Formulation, supply chain and logistics

- Aseptic fill and finish
- Cryopreservation
- Cold chain supply logistics
- Clinical trial support
- Global distribution





View complete infographic

FDA guidance for industry on characterization requirements for gene therapy products, focus on structure and characteristics of the product, process related impurities, and product related impurities.

Check out this new infographic to discover optimized workflows utilizing leading Thermo Scientific instruments, consumables, and software for AAV product and process characterization.

Learn more at **thermofisher.com/genetherapyanalysis**