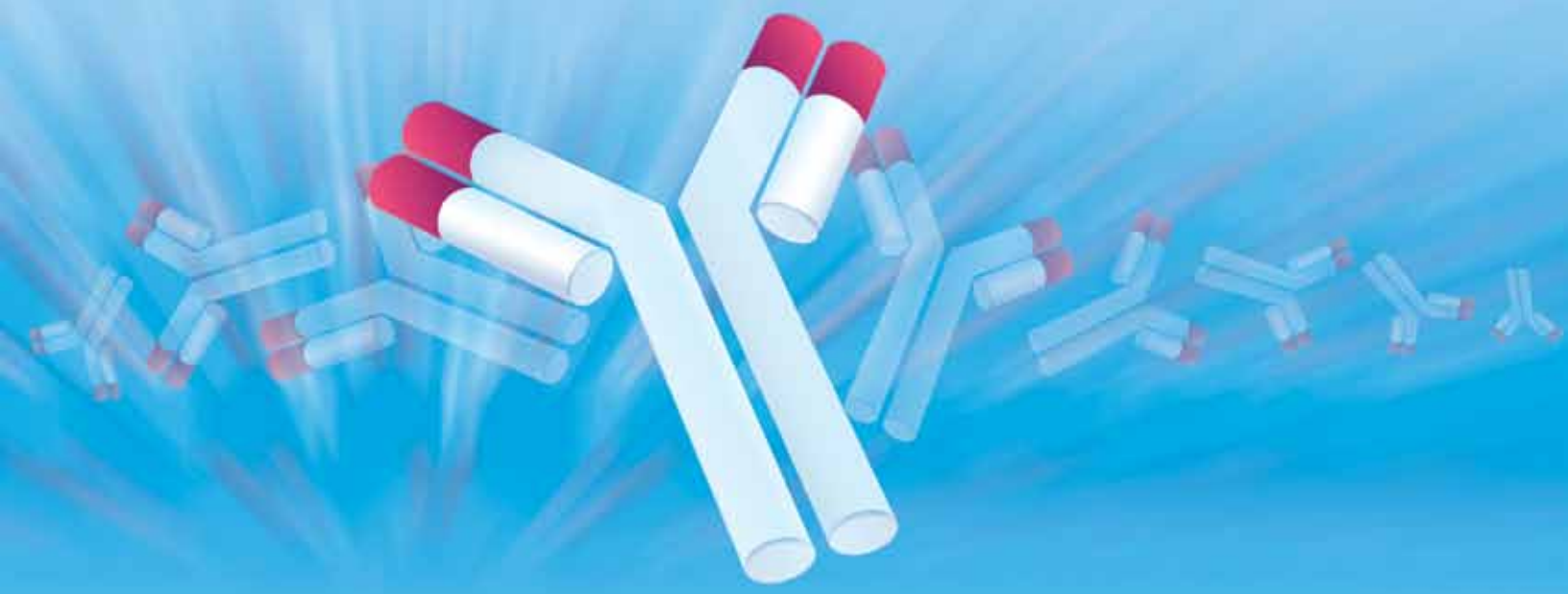
Monoclonal Antibody Characterization

Achieving Higher Throughput and Productivity

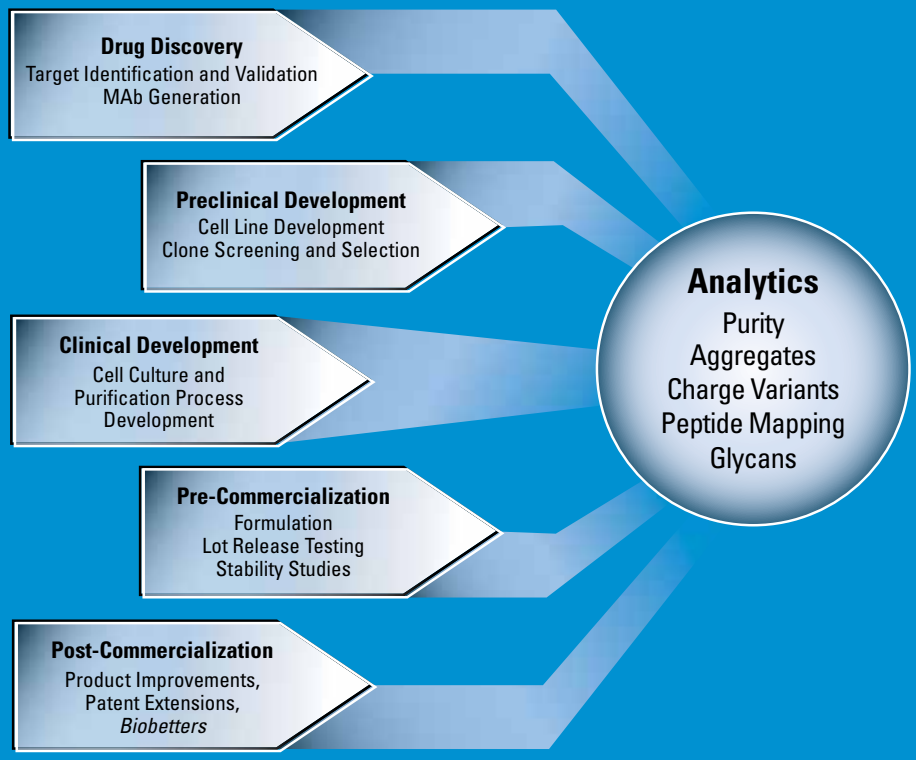


Dionex Solutions to Accelerate Monoclonal Antibody R&D and Characterization

The throughput and productivity challenge

- Increasing number of MAb candidates entering the clinical pipeline
- Advances in automation in upstream processes like cell culture and purification process development, and formulation screening
- Strict Quality-by-Design (QbD) guideline requiring enhanced antibody characterization

All result in a large increase in sample requests for characterization.



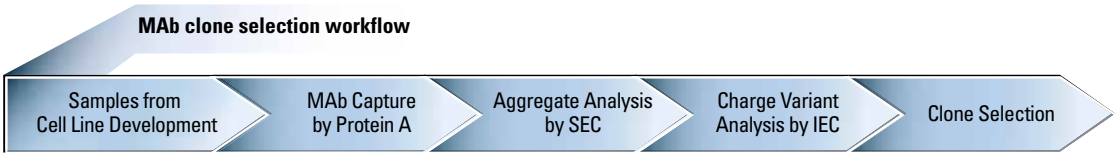
The analytical solutions

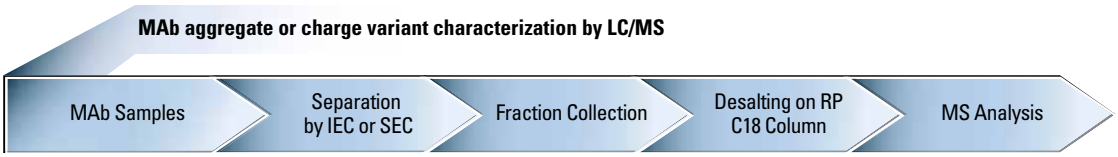
Dionex provides best-in-class analytical solutions for MAb therapeutics, improving throughput and productivity, thus reducing time to market.

- Best-in-class columns for high-resolution and high-throughput characterization using ion-exchange chromatography (IEC) and size-exclusion chromatography (SEC)
- MAb characterization platforms to increase sample throughput and streamline multistep workflows
- Powerful and flexible Chromeleon® Chromatography Data System software
- Innovative UltiMate® 3000 RSLC system for UHPLC solutions for fast MAb characterization and peptide mapping
- High performance MAb glycan analysis

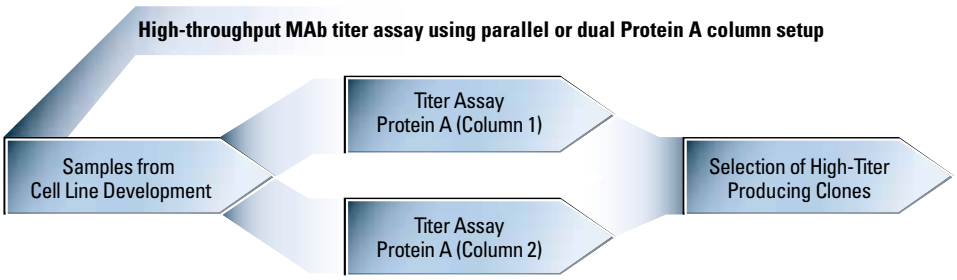
Whatever Your Workflow, You Can Achieve Higher Throughput and Productivity

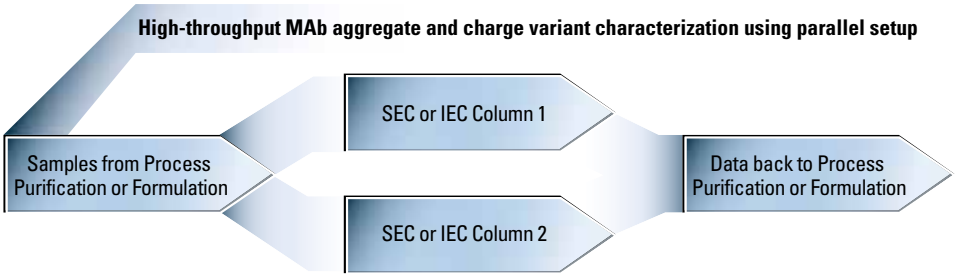
Multistep MAb characterization workflows

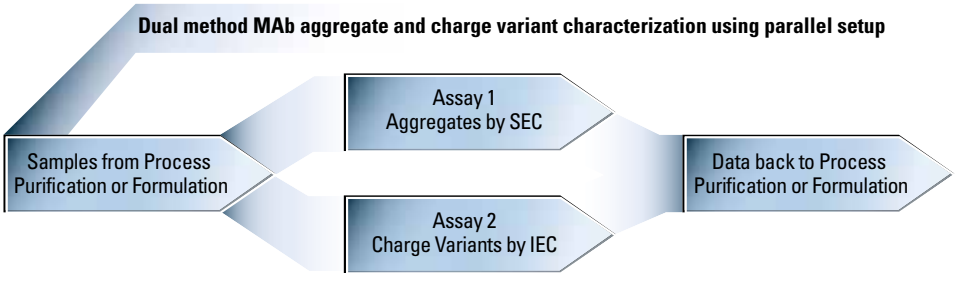




High-throughput MAb characterization workflows







The Dionex MAb Characterization Platform—

UltiMate 3000 MAb Analysis Platform with dual ternary gradient pumps and autosampler with integrated fraction collector.

UltiMate 3000 MAb fully biocompatible platform

- Titanium pumps; PEEK™ fluidics, valves and injection needle
- Full compatibility with all biological buffers
- Prevents iron poisoning of columns
- Maintains protein modifications
- Less system corrosion and maintenance

Dual gradient pump design: 2-in-1 system

- Multistep Automation: allows automation of two or more methods like Protein A capture, SEC aggregate, and IEC charge variant analysis
- Tandem Analyses: shortens run times by utilizing the power of off-line column regeneration
- Parallel LC: double throughput for productivity, cost, and space saving

Autosampler with fraction collection and re-injection

- Dual valve design allows injection, fraction collection, and re-injection
- Optimized for automated workflows like:
  - Automated multistep workflow automation
  - Protein purification
  - Sample fractionation and desalting prior to MS detection
  - Sample derivatization like digestion or neutralization between multistep separations

For Your Throughput and Productivity Needs

Chromeleon Chromatography Data System (CDS) software

Features of Chromeleon CDS software:

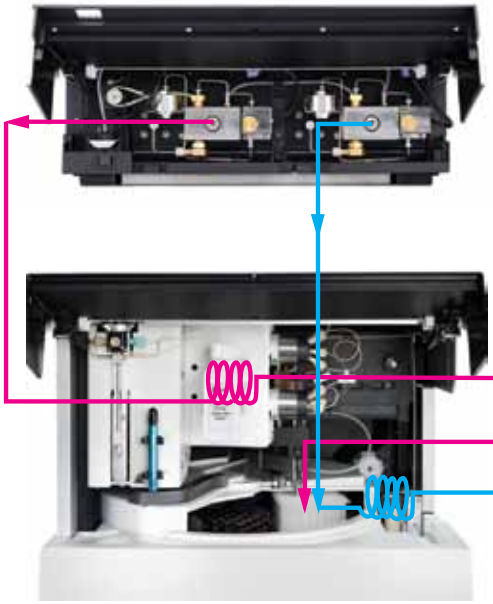
- Automated peak integration
- Automated control of fraction collection into autosampler
- Automated method template and sequence generation for multi-dimensional workflows
- Automatic rejection of samples based on set criteria e.g., automated rejection of clones with low antibody titer
- Dilutions or variable injection volumes for the next steps
- One-click report generation
- Validation report templates and sequences for increased automation of method validation

Fully integrated solutions for R&D, analytical method development and QA/QC.

Boost Productivity with Automated Multistep MAb Capture and Characterization

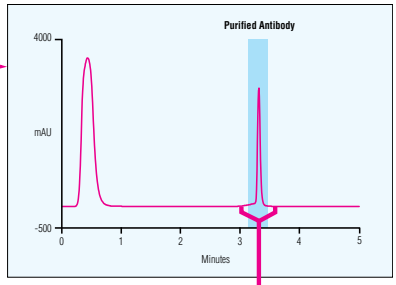
Automate your multistep MAb capture and characterization—reduce hands-on time and increase productivity

Dual Ternary Gradient Pump. 2-in-1 Pump Design.

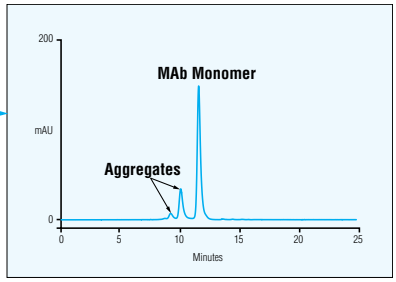


Autosampler with Injection, Fraction Collection, and Re-Injection. Two sampling technologies in one.

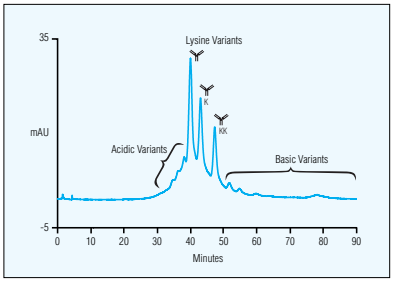
Step 1: Protein A capture of IgG (MAb) and automated fraction collection.



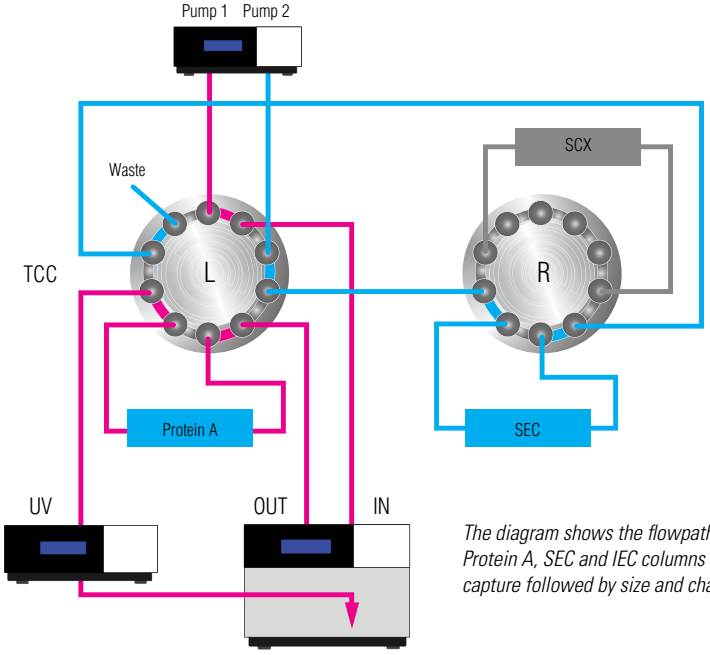
Step 2: SEC aggregate analysis.



Step 3: IEC charge variant analysis.



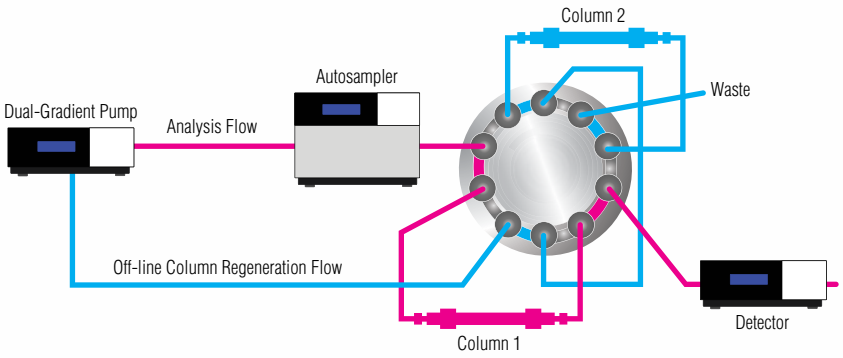
The Multistep MAb Analysis Platform allows the purification and analysis of hundreds of MAb samples. Cell culture fluid samples containing antibodies are injected onto a Protein A column for antibody recovery, then the purified antibody samples are automatically injected, first onto the SEC column, and then onto the IEC column—fully automated.

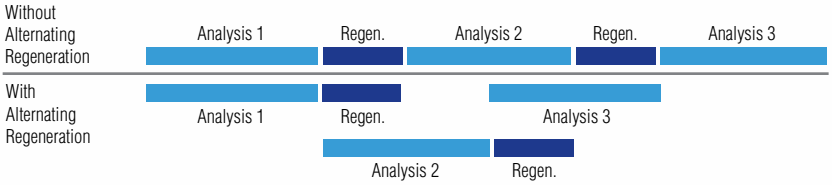


The diagram shows the flowpath configuration of the Protein A, SEC and IEC columns for fully automated MAb capture followed by size and charge characterization.

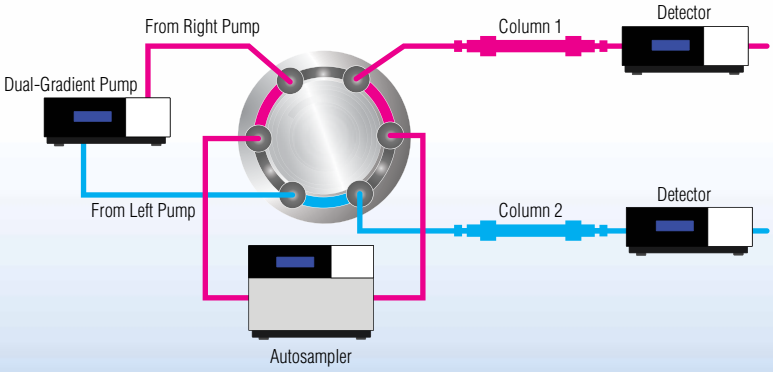
Accelerate Product Development by Increasing Sample Throughput

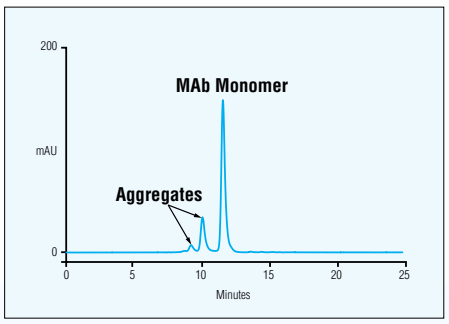
Analyze over a 1000 MAb samples a day by utilizing the power of off-line column regeneration

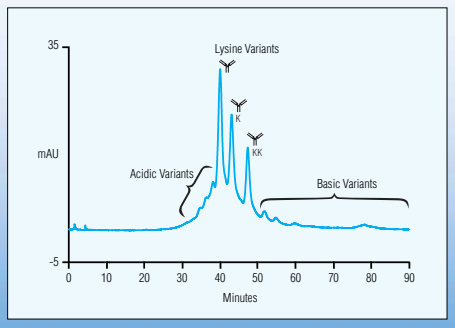




With the same samples perform multiple separation steps including SEC and IEC in a parallel configuration







Monoclonal Antibody Characterization Using

Charge Characterization with IEC

- Glycosylation
- Sialylation
- C-Terminal Lysine
- Deamidation
- Glutamine cyclization
- Maleuric acid adduct
- Oxidation
- Cysteinylation
- Disulfide related
- Succinimide
- Isomerization

Column

ProPac® WCX-10 (4 × 250 mm)
ProPac WCX-10HT (4 × 50 mm)

NEW!

MAbPac™ SCX-10 (4 × 250 mm)
MAbPac SCX-10 (4 × 150 mm)
MAbPac SCX-10HT (4 × 50 mm)
MAbPac SCX-10 for LC/MS (2 × 250 mm)

Hydrophobicity Characterization with HIC

- Isomerization
- Succinimide
- Oxidation
- Amidation
- Aggregation
- Clipping

Column

ProPac HIC (4.6 × 250 mm)
ProPac HIC (4.6 × 100 mm)
ProPac HIC-10 (2.1 × 100 mm)
ProPac HIC-10 (7.8 × 75 mm)

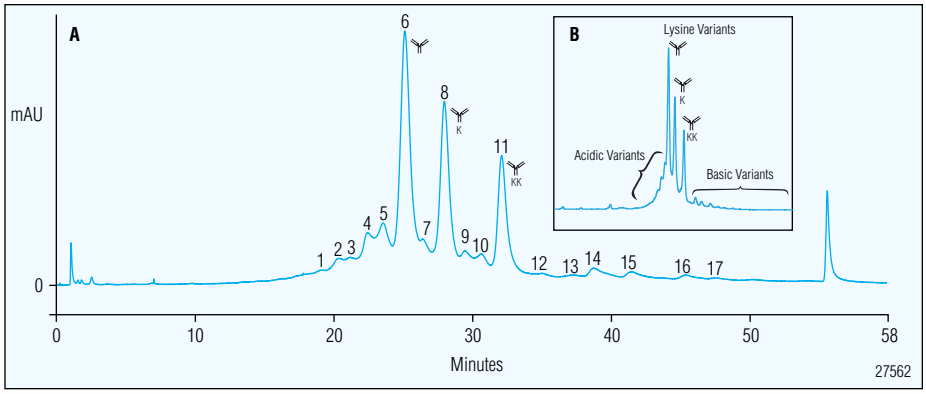
Size Characterization with SEC

- Monomers, aggregates, and fragments
- Under non-denaturing conditions using both high- and low-salt mobile phases and volatile eluents for LC/MS

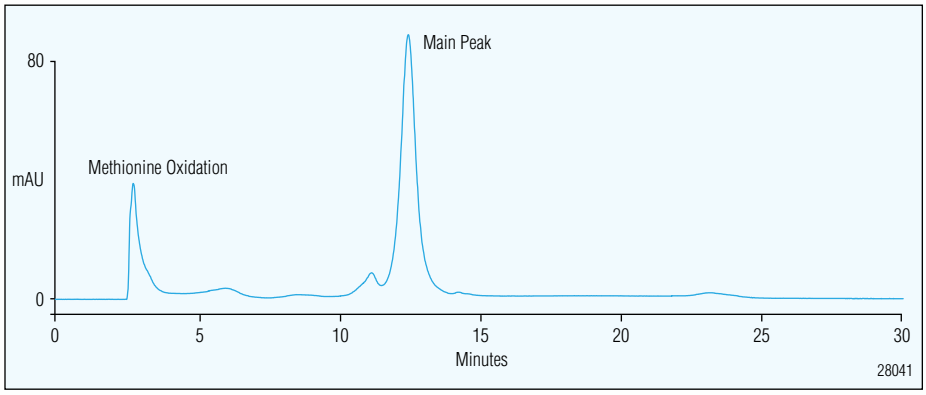
Column

MAbPac SEC-1, 5 µm, 300 Å (4.0 × 300 mm)
MAbPac SEC-1, 5 µm, 300 Å (4.0 × 150 mm)

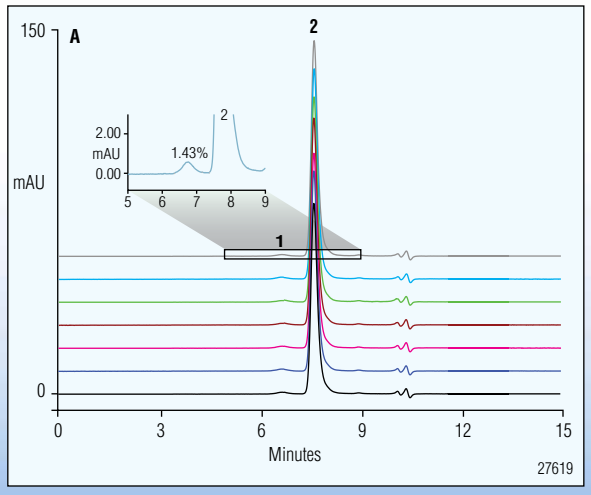
High-Resolution Columns



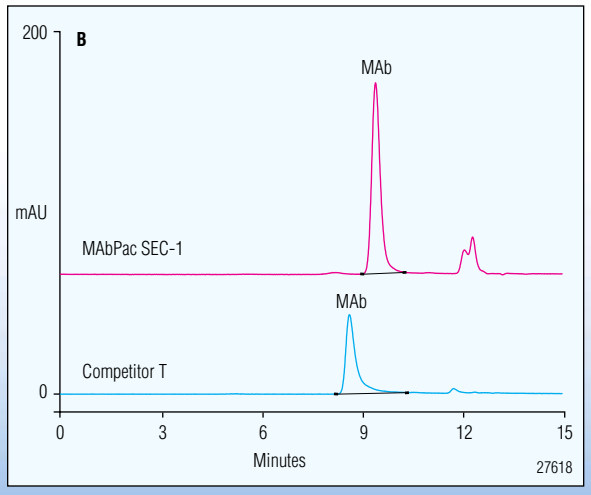
A. MAbPac SCX-10 column is the next generation IEC column for MAb charge characterization from Dionex.
B. ProPac WCX-10 column (inset) is the industry gold standard for charge variant characterization.



ProPac HIC-10 column - Separation of populations of MAb variants using hydrophobic interaction chromatography (e.g., methionine oxidation monitoring).



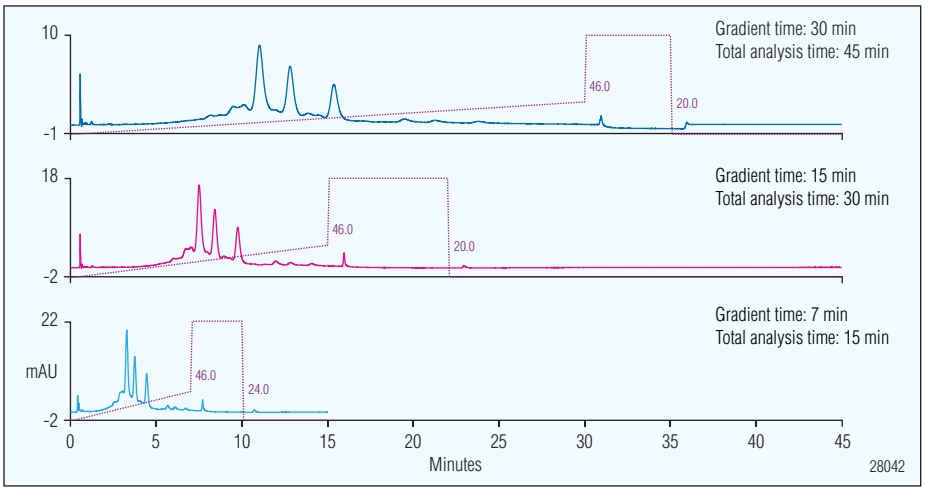
A. MAb analysis using the MAbPac SEC-1 column demonstrating excellent ruggedness.



B. MAb analysis in volatile buffer for LC/MS—MAb Pac SEC-1 vs the competitor's column.

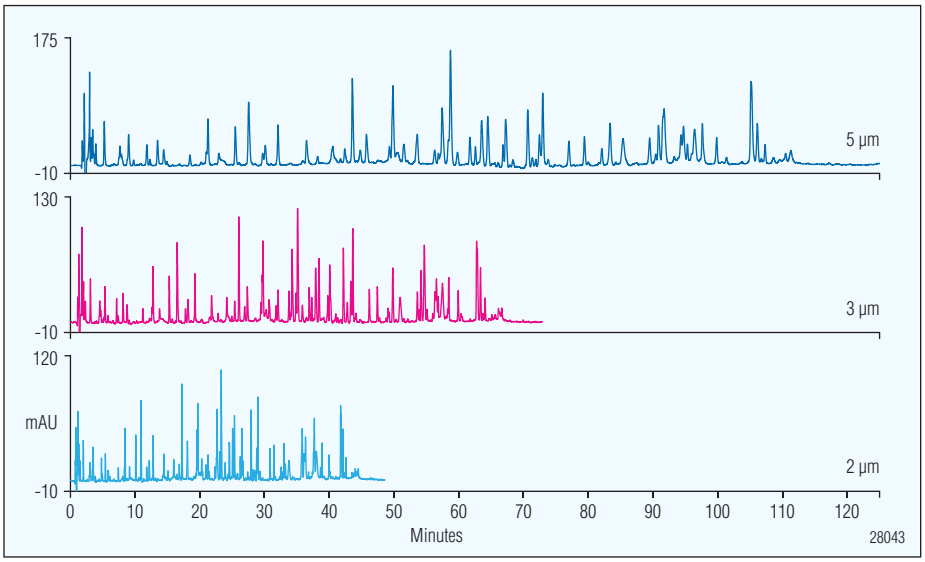
Fast MAb Characterization and Peptide Mapping

Method acceleration using the new MAbPac SCX-10 column, 4 × 150 mm



Elution profiles of a MAb sample from a 4 × 150 mm MAbPac SCX-10 column with different gradient elution times. The gradient time and total analysis time is indicated in the chromatogram.

Fast MAb peptide mapping—application of UHPLC and reduced particle size



Performing peptide mapping with smaller particle columns allows the user to achieve identical resolution in less time. Here a method transfer was performed using 2.1 × 100 mm columns packed with 5, 3, and 2 µm particles respectively on the UltiMate 3000 Rapid Separation LC (RSLC).

The Preferred and Proven Platform for MAb Glycan Characterization

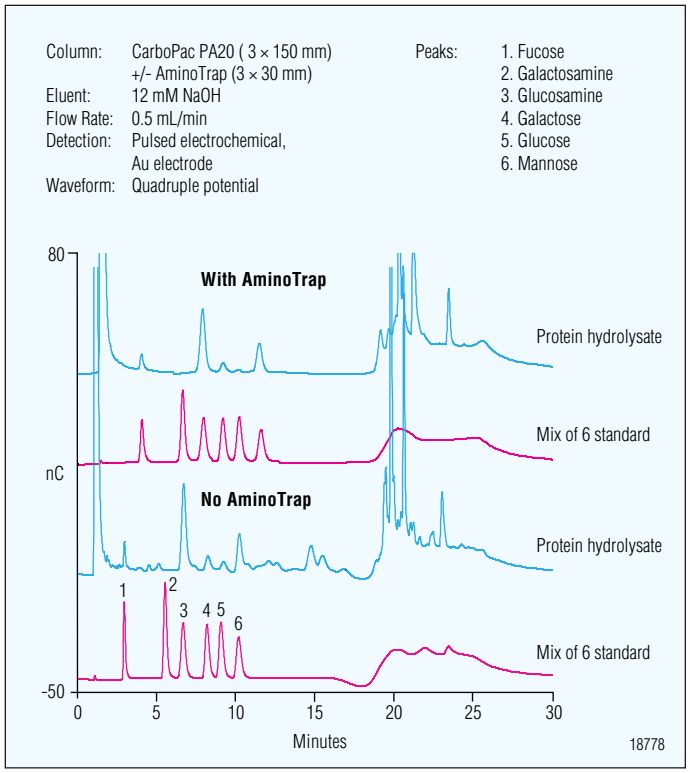
MAb glycosylation—why measure it?

- Increasing relevance–biosimilars
- Glycosylation can affect:
  - Biological activity
  - Pharmacokinetics and clearance *in vivo*
  - Stability
  - Immunogenicity
- Analysis of glycosylation is important to:
  - Meet regulatory requirement
  - Ensure product consistency
  - Develop new generation drugs with modified glycosylation

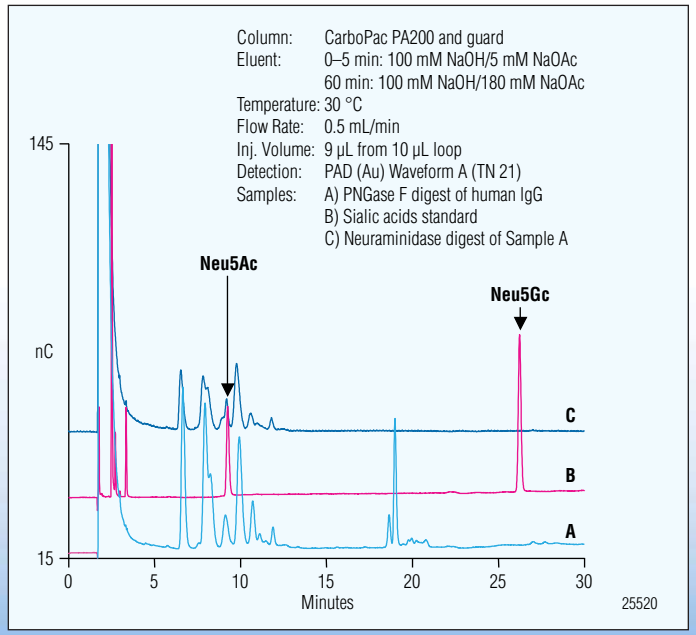
Dionex solution

- High Performance Anion-Exchange with Pulsed Amperometric Detection (HPAE-PAD)
  - The workhorse in carbohydrate analysis
  - High Sensitivity—0.1 to 1 pmole detection limits
  - Label-Free—No derivatization necessary

ICS-5000—New capillary system for carbohydrate analysis.



Profiling MAb hydrolysate on the CarboPac® PA20 column, with and without an AminoTrap™ precolumn.



Monitoring release of sialic acids from human IgG N-linked oligosaccharides by HPAE-PAD.

Selected Peer Reviewed Publications

1. Lyubarskaya, Y.; Houde, D.; Woodard, J. Murphy, D.; Mhatre, R. Analysis of Recombinant Monoclonal Antibody Isoforms by Electrospray Ionization Mass Spectrometry as a Strategy for Streamlining Characterization of Recombinant Monoclonal Antibody Charge Heterogeneity. *Anal. Biochem.* **2006,** *348,* 24–39.
2. Vlasak, J.; Ionescu, R. Heterogeneity of Monoclonal Antibodies Revealed by Charge-Sensitive Methods. *Curr. Pharm. Biotechnol.* **2008,** *9,* 468–481.
3. Valliere-Douglass, J.; Wallace, A.; Balland, A. Separation of Populations of Antibody Variants by Fine Tuning of Hydrophobic Interaction Chromatography Operating Conditions. *J. Chromatogr., A* **2008,** *1214,* 81–89.
4. Decrop, W.; Gendeh, G.; Swart, R. Development of an Automated Method for Monoclonal Antibodies Purification and Analysis. *Chromatography Today*, Jun 2009, 8–10.
5. Farnan, D.; Moreno, G.T. Multiproduct High-Resolution Monoclonal Antibody Charge Variant Separations by pH Gradient Ion-Exchange Chromatography. *Anal. Chem.* **2009,** *81*(21), 8846–8857.
6. Farnan, D.; Moreno, D.T.; Stults, J.; Becker, A.; Tremintin, G.; van Gils, M. Interlaced Size Exclusion Liquid Chromatography of Monoclonal Antibodies. *J. Chromatogr., A* **2009,** *1216*(51), 8904–9.
7. Rea, J.C.; Moreno, G.T.; Lou, Y.; Parikh, R.; Farnan, D. High-Throughput Multi-Product Liquid Chromatography for Characterization of Monoclonal Antibodies. *BioPharm International* **2010,** *23,* 44–51.
8. Grey, C.; Edebrink, P.; Krook, M.; Jacobsson, S.P. Development of a High Performance Anion Exchange Chromatography Analysis for Mapping of Oligosaccharides. *J. Chromatogr., B Anal. Technol. Biomed. Life Sci.* **2009,** *877*, 1827–1832.

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MAb Solutions

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