BIOPROCESS WORKFLOW SOLUTIONS

FROM START TO FINISH

bio-techne®

CONTENTS

BIO-TECHNE

Biotherapeutics save and improve lives. As the pace of approvals increases, so does the pressure on makers of new biologics to complete their development processes faster and more efficiently.

The best designed biologics manufacturing processes are efficient and reproducible. Streamlining the many steps in process development translates to less time required to get your product to market. We'll help you test all your samples during development to save you time and money downstream. We'll also help you confirm the integrity of your final products.

Our analytical instruments are automated, high-throughput, fast, and easy to use. They're extremely sensitive and reliable and will give you confidence for resolving difficult protein analysis questions.

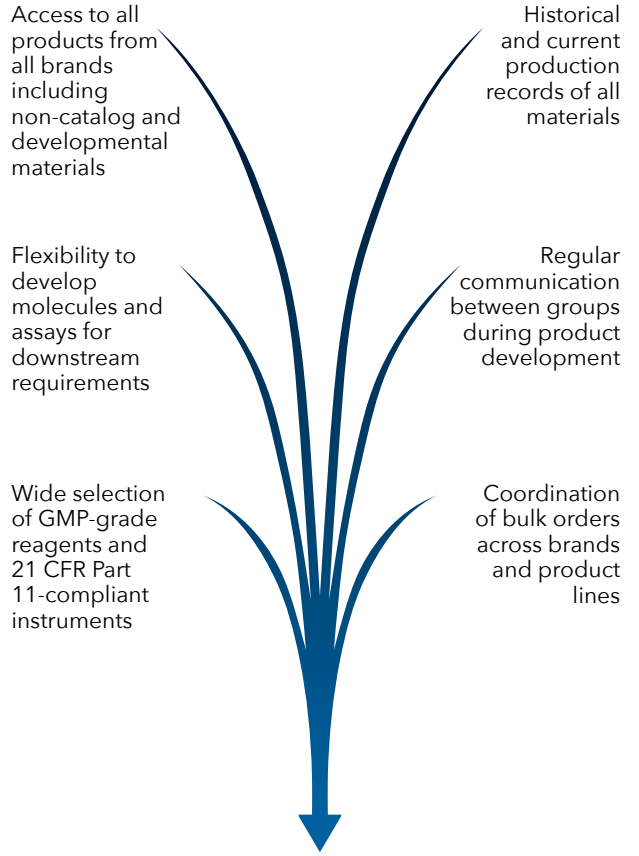
At Bio-Techne, we cover all the angles of process optimization and product characterization:

- Product purity measurement during process optimization
- Boosted cell culture yield with GMP-grade proteins and small molecules
- Functional characterization of your product's biological activity
- Analysis of contaminants, particulates, and protein fragmentation
- Charge and size heterogeneity analysis to establish process consistency
- Custom services with flexibility to meet your assay requirements

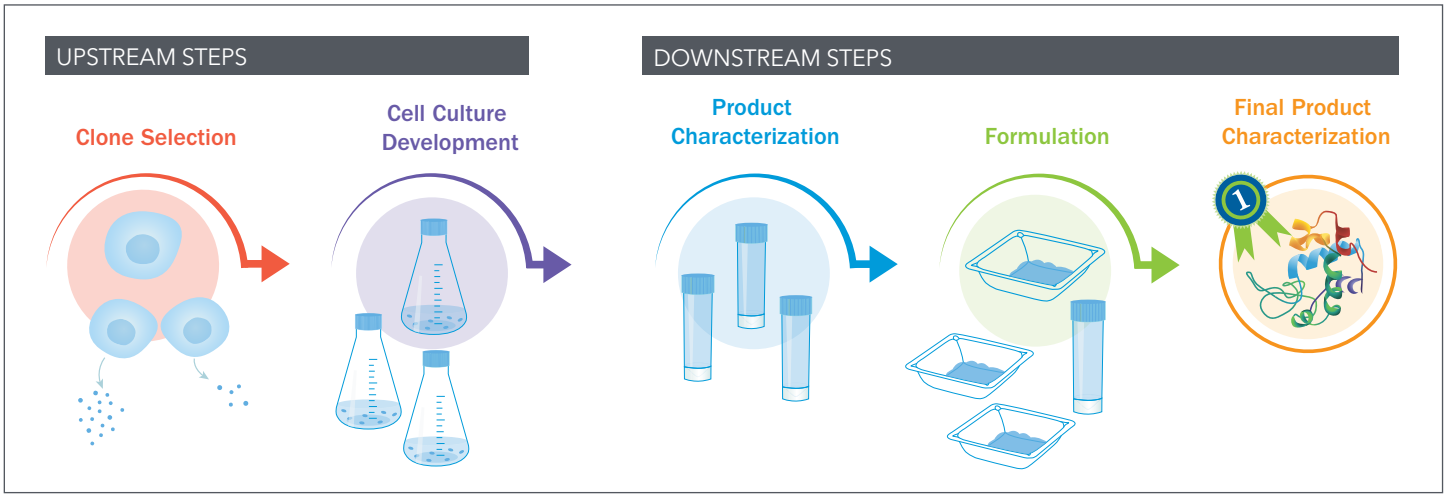
As you design, optimize, and implement your bioprocess, you can rely on us to support every step of way toward getting your therapeutic to the patients that need it!

Explore our Bioprocessing resources.

BENEFITS OF CHOOSING BIO-TECHNE



All this adds up to getting better results in the long run. We'll help you build a more efficient and reliable process!



OUR CAPABILITIES FOR BIOPROCESSING

SIMPLE WESTERN

The Simple Western™ family is made up of automated, capillary-based immunoassay platforms that combine the power of CE-SDS or cIEF with the sensitivity of immunodetection, enabling size- and charge-based screening of complex sample types.

- Separate and analyze proteins by either immunoassay or total protein content, from 2 kDa to 440 kDa or from a pI of 3 to 10.
- Analyze crude or purified samples for proteins of interest or biocontaminants.
- Quantitate expression levels, isoform distribution, and fragmentation in a gel- free, blot-free format.
- Choose from five instruments of differing throughput and separation mode options.

WATCH JESS VIDEO

MAURICE

Maurice is a capillary electrophoresis platform that automates protein profiling by size or charge. Maurice platforms employ pre-assembled cartridges and feature onboard sample mixing.

- Maurice streamlines cIEF and CE-SDS method development and data analysis for proteins, monoclonals, ADCs, and vaccines.
- Eliminates cross-contamination with separate cIEF and CE-SDS fluid paths.
- Five different instruments to give you choices.
- Whole column imaging by absorbance or native fluorescence.
- cIEF charge assay: 100 samples per run at 6 to 10 minutes each.
- CE-SDS size assay: 48 samples per run at 25 to 35 minutes each.

WATCH MAURICE VIDEO

OUR CAPABILITIES FOR BIOPROCESSING (CONTINUED)

MILO

Single-Cell Western on Milo™ analyzes individual cells to demonstrate monoclonality. Single-Cell Westerns can investigate protein expression and identity in just ~1,000 individual cells in a single run.

- Multiplex up to 12 proteins simultaneously including multiple isoforms.
- Ensure that your preliminary and production clones are truly clonal. Confirm that the expression level of your product is consistent across cells in your culture.
- Microscale immunoassays on ~1,000 cells in parallel.
- One-minute SDS-PAGE separation on each single-cell in your lysate.
- Chip data acquisition by InnoScan® microarray scanner

WATCH MILO VIDEO

MICRO-FLOW IMAGING

Micro-Flow Imaging™ (MFI) analyzes protein aggregation for stability measurements following stress testing. Up to 90 samples/run, and up to 150 µL/minute at 900,000 particles/mL.

- Image-based analysis of subvisible particle morphology.
- Differentiation of aggregates, silicone oil droplets, and bubbles.
- Autosampler for up to 90 samples per run.
- High-resolution images with 85% sampling efficiency.

WATCH MFI VIDEO

OUR CAPABILITIES FOR BIOPROCESSING (CONTINUED)

SIMPLE PLEX™ ASSAYS ON ELLA

Automated ELISAs detect fragments, oligomers, and host cell proteins with low assay CVs. Single or multianalyte cartridge format options available.

- Cartridge-based immunoassays with 4 to 5 log dynamic range.
- Up to 72 samples per run with a 60-minute time to result.
- Samples run in individual channels to eliminate cross-reactivity.
- Sub-picogram detection sensitivity

WATCH ELLA VIDEO

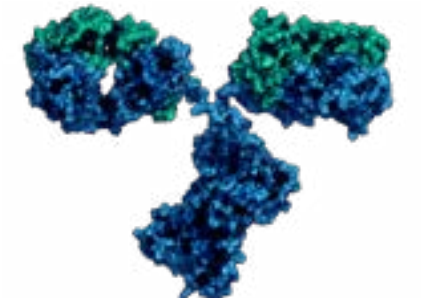
REAGENTS AND CELL CULTURE MEDIA

GMP-GRADE PROTEINS

- Nearly 50 available, including LR3, IGF-I, EGF, FGF basic, and PDGF-BB
- View all GMP proteins
- Certificate of Analysis specifications
- GMP Quality Policy and Regulatory Support

RESEARCH-GRADE PROTEINS

- Glycosidases, glycosyltransferases, and proteoglycans
- Hundreds of bioactive growth factors and cytokines
- Bulk quantities and custom formulations
- Custom protein services



ANTIBODIES

- Recombinant monoclonal antibodies for Ig fragment requirements
- Extensive selection of top quality off-the-shelf antibodies
- Bulk quantities and custom formulations
- Custom antibody services

SMALL MOLECULES AND PEPTIDES

- GMP-grade small molecule production
- Agonists, antagonists, and enzyme inhibitors
- Custom synthesis of small molecules and peptides

REAGENTS AND CELL CULTURE MEDIA

For more information:
Custom Reagents & Contract Services.

CELL CULTURE

- Media and supplements
- Specialty serum-free media
- GMP-grade media production
- Antibiotics and antifungals
- Serum lot reserve and matching
- Basement membrane matrices
- Extracellular matrix proteins
- Custom cell culture media manufacturing and services

IMMUNOASSAYS

- Quantikine® ELISA kits
- Luminex® and Simple Plex™ multianalyte detection assays
- Custom assay services

BIOASSAYS

- Cell-based bioactivity assays
- Cell-based binding assays
- Bioactivity testing services

ENZYMATIC ASSAYS

- Glycotransferases and glycosidases
- Proteases and inhibitors
- Kinases and phosphatases
- Custom assay development

CUSTOM REAGENTS & SERVICES

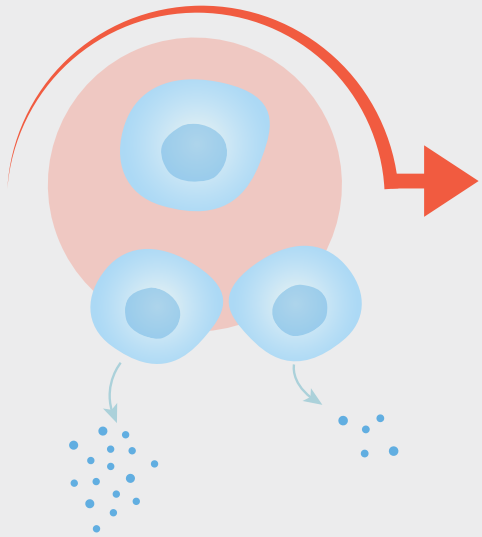
CAPABILITIES

- Reagents and cell culture media
- Immunoassays and bioassays
- Bulk quantity agreements and custom formulations
- Production capacity and supply chain reliability

EXPERIENCE AND RELIABILITY

- Dedicated product managers and expert scientists
- Project consultations
- Documented milestones
- Communication throughout a project
- Delivery of comprehensive reports

CLONE SELECTION



You need to screen preliminary clones, identify a production clone, and validate the clonality of your master cell bank. Our automated, high-throughput assay platforms let you do this by quickly analyzing complex cell culture samples for the highest yield of your biologic.

ARTICLES IN THIS CHAPTER:

PICK THE RIGHT CLONE FASTER

Which clones to grow, which clones to leave behind? Make fast, accurate decisions with Simple Western™ size analysis of proteins from 2 kDa to 440 kDa with picogram-level sensitivity and just 3 µL to 5 µL of starting material required. In a hurry? Jess runs 25 samples in 3 hours flat. If you have lots of clones to screen, Sally Sue™ and Peggy Sue™ run 96 samples overnight.

A key factor in reducing the production costs of biopharmaceuticals is the development of cell lines or bacterial cultures producing a high yield of product. Such phenotypic screens will commonly utilize a variety of technologies, including affinity assays, ELISA, cDNA sequence analysis, CE-SDS, size-exclusion chromatography (SEC), iCIEF, and Western blotting. These technologies assist with selecting clones for optimal manufacturing characteristics (robust cell growth and scalability) and product qualities (three-dimensional conformation and stability). Efforts to improve cell line selection have focused on the introduction of screens early in development that are predictive of manufacturing performance. Early stage development screening often requires analyzing small volumes of crude mixtures. Simple Western is a capillary-based immunoassay, requiring no purification steps, even with complex mixtures. In addition, Simple Western size-based assays have low sample volume requirements (3–5 µL) and a broad dynamic range for protein detection. This allows clones to be screened earlier, often with minimal dilution compared with traditional Western blotting approaches. Simple Westerns also provide confirmation of your protein's molecular weight, unlike other immunoassays such as ELISA. Automation of the entire process delivers results in 24 hours or less compared with 1 to 3 days and the significant manual labor that comes with Western blotting.

Scientists in Vaccine Analytical Development at Merck Research Labs, West Point used Simple Western size assays for clone selection (**FIGURE 1**). The approximately 95 kDa Binary toxin B (proCDTb) is one of four protein antigens from the Clostridium difficile vaccine. This Merck screen compared seven different clones (C1–C7) for yield and product quality. Clone 4 did not express proCDTb, whereas clones 2 and 3 showed protein degradation. Samples from clones 5, 6, and 7 showed both protein degradation and aggregation. Clone 1 expressed protein of the expected molecular weight with no degradation or aggregation, making it the clear choice to move into cell culture development.

MEET SALLY SUE

MEET JESS

Explore our Bioprocessing - Clone Selection resources

Looking for pricing? Request a quote.

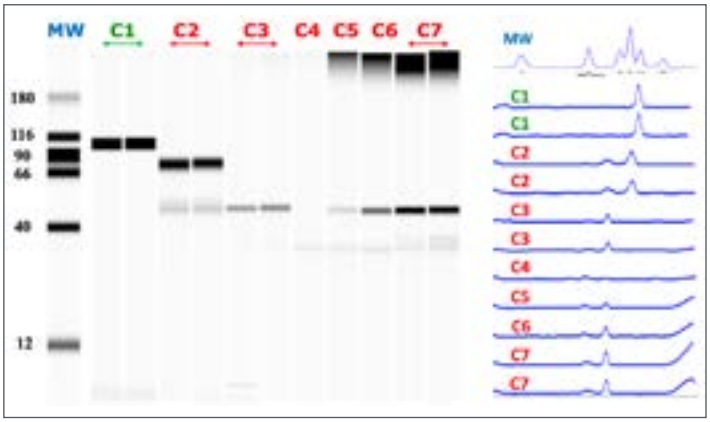


FIGURE 1: Seven different clones screened for proCDTb with the Simple Western Size Assay. Protein was detected in crude baculovirus lysate. Fragments and/or aggregation was observed in clones 2, 3, 5, 6 and 7 while no protein was observed in clone 4. Clone 1 expressed the expected protein of interest, making it the obvious candidate to move forward.

REFERENCES

1. Applications of an automated and quantitative CE-based size and charge western blot for therapeutic proteins and vaccines, RR Rustandi *et al*., *Capillary Electrophoresis of Proteins and Peptides*, New York: Springer, 2016; 197–219.

SIMPLIFY CHARGE CHARACTERIZATION IN COMPLEX CULTURES

Do a deep dive into charge heterogeneity with Peggy Sue's Simple Western™ charge assay. Got small sample volumes or starting materials? No problem. She uses as little as 0.05 µg/µL of protein in just 10 µL of sample and runs up to 96 samples in one experiment. Charge ahead and do more with Peggy Sue!

Characterizing charge heterogeneity of a biologic is critical for product safety, purity, and potency. Analysis of early stage development clones helps optimize the health and stability of the host cell lines and saves considerable time and effort downstream. The optimization process begins with the selection of a stable monoclonal cell line and establishment of cell banks, and it continues with the maintenance of an appropriate growth environment in the production bioreactor. Post-translational modifications such as glycosylation, oxidation, and deamidation often change the isoelectric point of the molecule. They can affect product safety and potency, making charge heterogeneity characterization a critical quality attribute for biotherapeutics.[1] Simple Western charge assays have become a favored method due to their quantitative readout, reproducibility, automation, and ability to analyze complex samples of low concentration.

As part of a biosimilar development program at Merck, Peggy Sue was used to analyze samples from a heavily sialylated Fc-fusion protein, Enbrel (**FIGURE 2**). Charge profiles are shown for three biosimilar clones compared with the commercial product (Innovator). Variations in sialylation can affect the efficacy of glycoproteins, particularly antibodies, and can result from changing bioprocess conditions. Charge profiling with Peggy Sue helped streamline the evaluation of Pichia pastoris clone samples benchmarked against the charge variant profile of the innovator. This demonstrates the value of Simple Western charge assays for rapid screening of low volume cell culture samples prior to purification. This method can also be applied to other heavily sialylated therapeutic proteins such as erythropoietin (EPO).[2]

MEET PEGGY SUE

Watch Milo video

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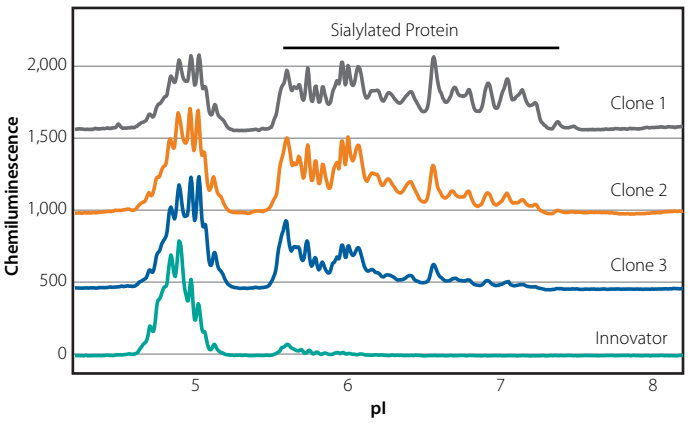


FIGURE 2: Charge-based Simple Western analysis with Peggy Sue during biosimilar development of Enbrel, produced from Pichia pastoris. Charge profiles are shown for three clones and compared with the innovator. All final concentrations of sample were 0.5 µg/mL.

REFERENCES

1. Post-translational structural modifications of immunoglobulin G and their effect on biological activity, LR Hmiel *et al.*, *Anal Bioanal Chem,* 2015;407(1):79–94.

2. Applications of an automated and quantitative CE-based size and charge western blot for therapeutic proteins and vaccines, RR Rustandi *et al.*, *Capillary Electrophoresis of Proteins and Peptides*, New York: Springer, Humana Press p202, 2016; 197–219.

EASY VERIFICATION OF CLONE MONOCLONALITY

Your clone should stand out from the crowd. With Single-Cell Westerns on Milo™, you get protein expression information for over 1,000 individual cells simultaneously. That means, for the first time, you can easily check for clone monoclonality.

Verification of a cell line's monoclonality is crucial for cell line generation. Regulatory guidelines for cell line development are increasingly requiring heightened scrutiny of the cloning method used.[1] Even if monoclonality is achieved, significant changes in protein expression can occur over time and with increasing passage number. Product heterogeneity can result from incorrect translation of the target protein, post- translational and enzymatic modifications, or changes in sample processing and storage.[2,3] Peptide mapping by mass spectrometry is used to characterize protein sequence variants, but detection and characterization of trace variants remains a significant technical challenge.[4] Conventional Western blotting techniques only provide information about the overall cell population in a culture sample. Flow cytometry can resolve single-cell heterogeneity, but results are often confounded by off-target binding or detection of target variants and isoforms. In contrast, Single-Cell Westerns on Milo are SDS-PAGE separations of 1,000 single-cells in parallel and can distinguish off-target binding and protein variants/isoforms, enabling verification of clone monoclonality for the first time.

In **FIGURE 3**, HeLa-GFP cells were analyzed with Milo and then mixed with an equal number of wild type HeLa cells. Quantitation of GFP+ cells on Milo is within 2.5% of the predicted value. In addition, expression heterogeneity in GFP+ cells can be quantified and tracked over time and passage number.

REFERENCES

1. Establishing clonal cell lines – A regulatory perspective, S. Kennett, 18th Symposium on the Interface of Regulatory and Analytical Sciences for Biotechnology Health Products, 2014.
2. Chromatographic and electrophoretic characterization of protein variants, K Ahrerand and A Jungbauer, *J Chromatogr*, 2006; B 841:110–122.
3. Commercial manufacturing scale formulation and analytical characterization of therapeutic recombinant antibodies, RJ Harris *et al*., *Drug Dev Res*, 2004; 61:137–154.
4. Low level sequence variant analysis of recombinant proteins: An optimized approach, A Zeck *et al*., *PloS One,* 2012; 7.7: e40328.

MEET MILO

Watch Milo video.

Explore our Bioprocessing - Clone Selection resources

Looking for pricing? Request a quote.

DID YOU KNOW?

If purifying your protein before clone selection testing isn't a hassle, you can use Maurice™ or iCE3™ for charge heterogeneity studies, too. They both use native fluorescence detection so you don't need antibodies, and they're a snap to use.

EASY VERIFICATION OF CLONE MONOCLONALITY (CONTINUED)

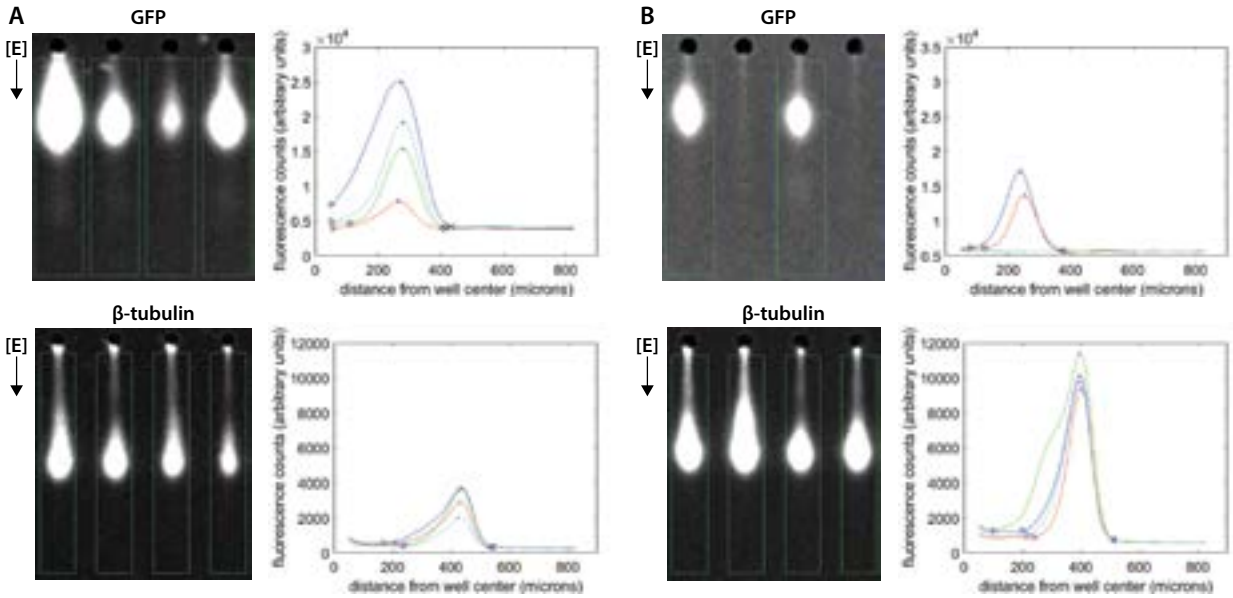


FIGURE 3: Monitoring GFP expression in single cells. A, top, left: four cells expressing GFP; bottom, left: same four cells expressing loading control β-tubulin. Corresponding electropherograms are shown on the right. B, top, left: two cells expressing GFP; bottom, left: β-tubulin in all four corresponding lanes indicates the presence of four cells, showing that only two of the four cells express GFP signal. Corresponding electropherograms are shown on the right.

ANTIBODY CERTIFICATION FOR SIMPLE WESTERN

Need a great antibody for your Simple Western™ immunoassay? Our scientists have certified over 1,300 R&D Systems® and Novus Biologicals® antibodies for Simple Western, and each of these antibodies is proven to give consistently strong results. We've even given them a badge of honor.

Simple Westerns make screening and optimizing antibodies for your targets easy by running independent Westerns via capillaries, allowing you to quickly assess multiple antibodies with varying concentrations in a single run.

Don't have enough time or resources to run Westerns? We can help you save time and money by letting us do the testing. If you have antibodies that work well for your purposes, we can let you know which of them plays well with Simple Western. We're all set up for this kind of screening to get you results faster and cheaper. If you can't find antibodies that work for you, our Custom Antibody Services can help you find a solution.

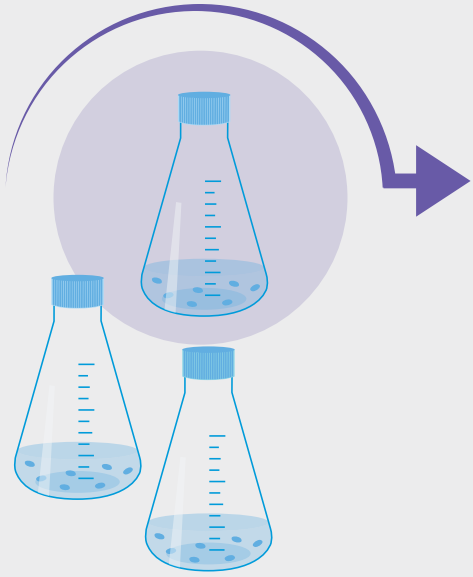
Explore our Bioprocessing - Clone Selection resources

Looking for pricing? Request a quote.

DID YOU KNOW?

Find Simple Western™ certified antibodies through R&D Systems, or visit the Novus Biologicals website to find all our antibodies certified for Simple Western™

CELL CULTURE DEVELOPMENT



The cells that produce your biologic need a welcoming environment to grow and do their work. You need to provide them with the best basal medium and supplements available while you optimize cell culture parameters. In order to develop the best cell culture for your process in a rapid and thorough manner, you also need quick turnaround, high-throughput assays of unpurified samples to check the yield and integrity of your product.

ARTICLES IN THIS CHAPTER:

RAMP UP CELL CULTURE GROWTH: CONDITION OPTIMIZATION

Got a lot of cell culture conditions to screen through? Simple Western™ size assays on Sally Sue and Peggy Sue let you separate and analyze proteins between 2 kDa and 440 kDa on up to 96 samples at a time. That means you can get through all your process and media conditions fast. Need absolute quantitation? Set up a standard curve and get quantitative results.

Production cell lines used for bioprocessing require well-defined culture conditions that optimize yield while maintaining product quality. Cell culture development can require months to evaluate the dozens of relevant variables. HPLC is a traditional method for determining protein and metabolite concentrations, but it's a slow serial technique, requires purified samples, and requires the use of organic solvents for reverse phase chromatography (RP-HPLC). Simple Western size assays provide the throughput, sensitivity, and specificity you need to quickly assess crude culture supernatants or cell lysates from multiple culture conditions.

Researchers at Merck Research Labs in West Point used Simple Western size assays to monitor monoclonal antibody production and quality during cell culture development. Samples were harvested on days 6 through14 and analyzed under reducing and non- reducing conditions (**FIGURE 4**). Protein production increased over time, with no evidence of fragmentation or inappropriate disulfide formation between HL-LC or HC- HC. The authors noted that the Simple Western size immunoassay is a fast, simple method for determining the time point of maximum protein concentration in cultures, and that this method is a valuable alternative to the protein A HPLC IgG titer assay commonly used to measure IgG production and product quality.[1]

Scientists from Merck also used a Simple Western size assay to measure binary toxin CDTa (C. difficile vaccine) protein titer in early cell cultures from different culture conditions (**FIGURE 5**). The high level of assay specificity enabled the group to measure the CDTa protein directly in cell lysates without the need for a purification step. They were also able to perform quantitative analysis of protein concentrations by setting up a five-point standard curve in the same experiment. Measured protein concentrations showed good correlation to values obtained by RP-HPLC with an R^2 > 0.967.

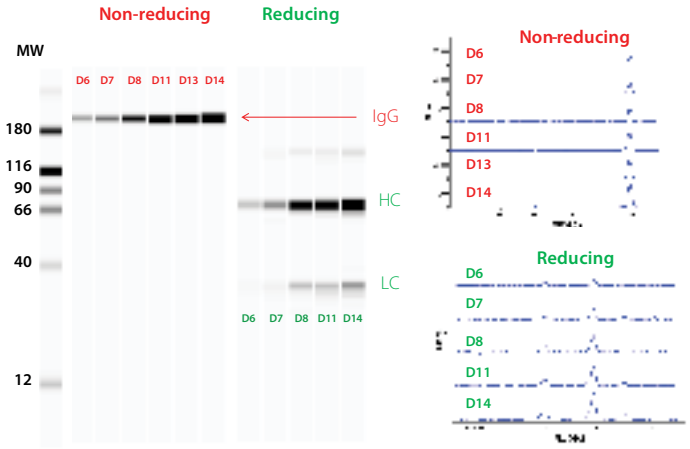


FIGURE 4: Simple Western size measurement of a monoclonal antibody in upstream production. Samples taken on days 6–14 were analyzed under non-reducing (left lane view of virtual gel image) and reducing (right lane view) conditions. Electropherograms of the same data are shown on the right. In addition to maximizing protein production, protein integrity was confirmed, and no clipping or misfolding between HL-LC and HC-HC was observed.

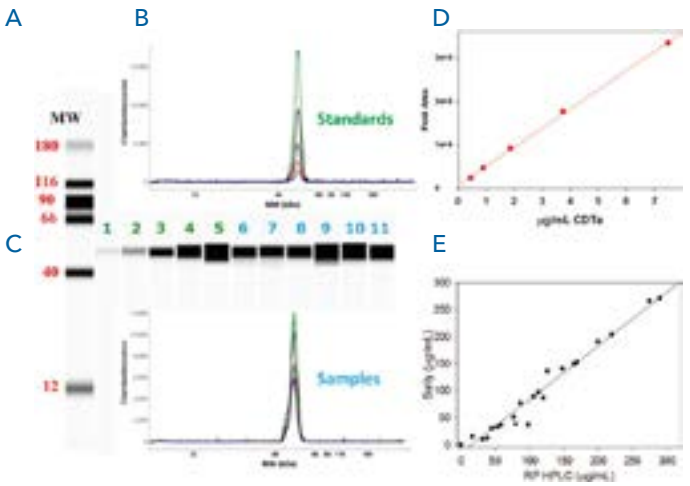


FIGURE 5: Simple Western measurement of a CDTa titer in various cell culture conditions. (A) Gel image of standard curve (lanes 1–5) and samples (lanes 6–11); (B) Electropherograms of standard curve in (A); (C) Electropherograms of samples in (A); (D) Linearity of standard curve from 0.45 to 7.5 µg/mL with R2 > 0.990; (E) CDTa titer correlation curve between Simple Western and the traditional RP-HPLC method with R2 > 0.967.

REFERENCES

1. Applications of an automated and quantitative CE-based size and charge western blot for therapeutic proteins and vaccines, RR Rustandi *et al*., *Capillary Electrophoresis of Proteins and Peptides*, New York: Springer, 2016; 197–219.

2. Quantitation of CRM197 using imaged capillary isoelectric focusing with fluorescence detection and capillary western, JW Loughney *et al*., *Anal Biochem*, 2017; doi 10.1016/j.ab.2017.06.013.

ANALYZE CULTURE CONDITION IMPACT ON CHARGE VARIANTS IN COMPLEX LYSATES

Need both size- and charge-based separation? Peggy Sue does both with the same throughput and speed, with only 5 µL to 10 µL of sample. And with the specificity of Simple Western™ immunoassays, you won't have to clean up your lysate before analysis either. Get a fresh perspective of your proteins with Peggy Sue!

Confirming consistent product quality of recombinant monoclonal antibodies (rmAbs) is often achieved by monitoring charge variation. Acidic charge variants in particular can affect biological activity of rmAbs. Ion exchange chromatography (IEX) and mass spectrometry are commonly used to monitor charge heterogeneity, but they're time consuming and require purified samples.[1] Traditional cIEF methods make this process easier and faster but still require sample purification before analysis. Peggy Sue analyzes charge heterogeneity in complex lysates, making it easier to monitor this critical quality attribute early in the cell culture development process.

In this example, Simple Western charge assays were run by scientists at Merck for monitoring the heterogeneity of a therapeutic rmAb during cell culture production. **FIGURE 6** illustrates an assay performed early in the process and shows an increase in acidic variants over the course of nine days.[2] The authors stated that having this information early in cell culture development is critical for managing harvest time and preventing an increase in acidic variants from affecting the performance of the molecule.

MEET SALLY SUE MEET PEGGY SUE

DID YOU KNOW?

With Peggy Sue you can analyze your protein of interest using both size and charge separation—one instrument answering different questions crucial for choosing an optimal culture!

REFERENCES

1. Elucidating the effects of pH shift on IgG1 monoclonal antibody acidic charge variant levels in Chinese hamster ovary cell cultures, P Xie *et al.*, *Appl Microbiol Biotechnol*, 2016; 100(24):10343–10353.
2. Applications of an automated and quantitative CE-based size and charge western blot for therapeutic proteins and vaccines, RR Rustandi *et al.*, *Capillary Electrophoresis of Proteins and Peptides*, New York: Springer, 2016; 197–219.

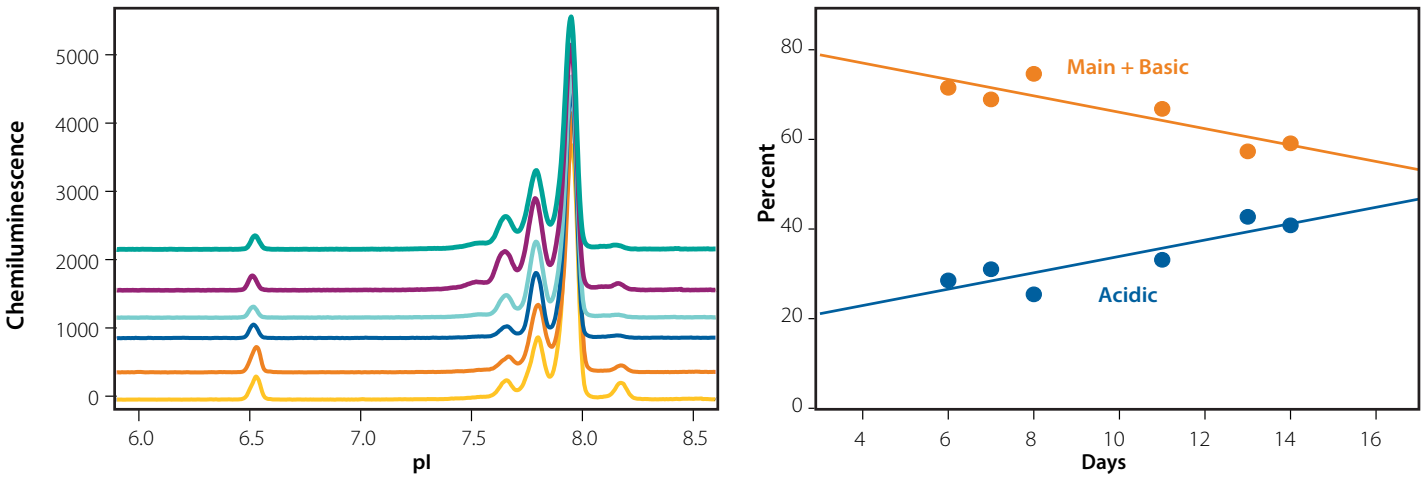


FIGURE 6: As part of biosimilar development of Enbrel® produced from Pichia pastoris, Peggy* was used to monitor charge heterogeneity. Electropherograms from day 6 to 14 show that the percent acidic variants increase during production (graph on right). The extra peak at pI = 6.5 is likely light chain, since there is always more light chain than heavy chain during cell culture production.

FASTER CHECKS FOR PROTEIN PURITY AND YIELD

Wes gives you size-based immunoassays and total protein data for 25 samples in just 3 hours. If you're using columns to purify your protein from crude lysates, you can do a quick check on overall binding and elution efficiencies with the Total Protein Assay, then flip to Simple Western™ size assays for product identity.

Simple Western assays enable high-throughput analysis for monitoring the product purification process. Product yield during cell culture can be quickly assessed by using UV absorbance to determine protein concentration, but this can't distinguish your protein from host-cell proteins, target molecule variants, or media components. ELISAs and traditional Western blots measure both protein purity and yield, but they can be tedious and labor-intensive. With multiple manual handling steps, errors and seemingly minor changes in the workflow can lead to significant data variability. Simple Western size assays help you identify molecule variants with the speed and consistency of automation.

Our scientists used Simple Western to monitor GSTDJ-1 protein production and purification. Samples were run in quadruplicate capillaries on Wes using the Total Protein Assay and the Simple Western size-based immunoassay. CVs for the GST-DJ-1 peak areas were all under 6.6%, demonstrating Wes's reproducible quantitation. Compass for Simple Western Software simplified obtaining quantitative data for peak area, % total area, and % CVs. In addition, purification samples were run on Wes using the Total Protein Assay as well as the size-based immunoassay with an anti-DJ-1 antibody (**FIGURE 7**). Just 0.5 µL to 2 µL was enough to assess total production and purification efficiency. The presence of GST-DJ-1 in the flow-through indicates an incomplete capture of product during column loading. Total protein and molecular weight information were provided in 6 hours, compared with the approximately 3 days needed to obtain just immunoassay data using traditional Western blotting. Precision and reproducibility of Simple Westerns are significantly better than with that achievable via Western blotting, too.

REFERENCES

1. Total Protein Analysis the Simple Western Way, ProteinSimple Application Note.
2. Characterization of a biopharmaceutical protein and evaluation of its purification process using automated capillary Western Blot, D Xu *et al*., *Electrophoresis*, 2015; 36:363–370.

Learn more about Simple Western™.

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Looking for pricing? Request a quote.

DID YOU KNOW?

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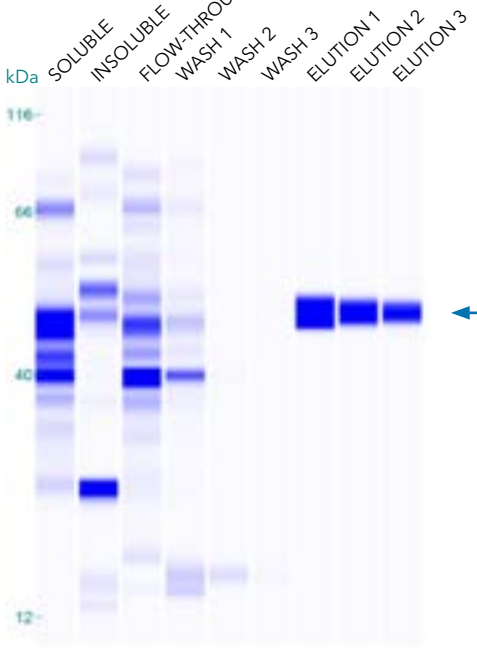
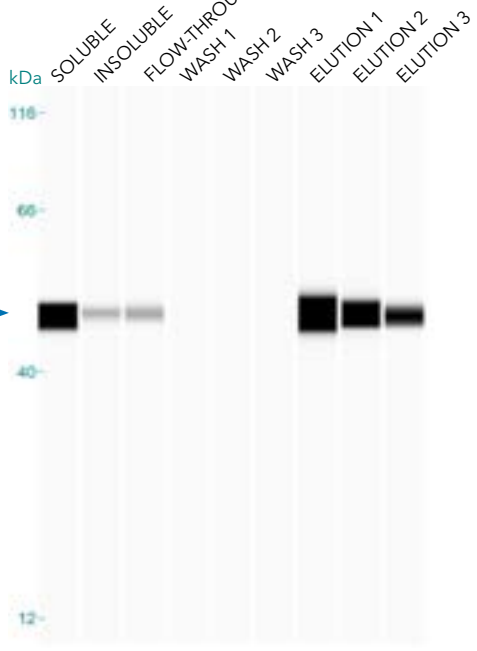
FASTER CHECKS FOR ROTEIN PURITY AND YIELD (CONTINUED)

A



GST-DJ-1

C



B

Sample	Name	MW (kDa)	Area	% Total
Soluble	GST-DJ-1	49	139152	29.38
Insoluble	GST-DJ-1	49	44245	14.56
Flow-through	GST-DJ-1	49	74161	15.61
Wash 1	GST-DJ-1	48	37747	14.94
Wash 2	GST-DJ-1	48	4404	12.99
Wash 3	GST-DJ-1	49	57	0.79
Elution 1	GST-DJ-1	49	127217	98.10
Elution 2	GST-DJ-1	49	88026	98.70
Elution 3	GST-DJ-1	49	68788	98.72

D

Sample	Primary	Name	MW (kDa)	Area
Soluble	anti-DJ-1	GST-DJ-1	49	27515
Insoluble	anti-DJ-1	GST-DJ-1	49	4033
Flow-through	anti-DJ-1	GST-DJ-1	49	4679
Wash 1	anti-DJ-1	GST-DJ-1	49	830
Wash 2	anti-DJ-1	GST-DJ-1	50	101
Wash 3	anti-DJ-1	GST-DJ-1	49	0
Elution 1	anti-DJ-1	GST-DJ-1	49	59966
Elution 2	anti-DJ-1	GST-DJ-1	49	24763
Elution 3	anti-DJ-1	GST-DJ-1	49	11670

FIGURE 7: Purification process monitoring. Samples were affinity purified using Glutathione Sepharose beads, screened with the Total Protein Assay (A), and analyzed with Compass software for Simple Western (B). Samples were then diluted 1:1000, probed with anti-DJ-1 antibody to specifically detect the GST-DJ-1 in the sample (C), and analyzed using Compass for Simple Western (D).

GMP-GRADE MEDIA COMPONENTS – PROTEINS, SMALL MOLECULES, AND MEDIA

When it comes to cell culture optimization, don't overlook factors that can boost your production cell line's growth and output. Maybe your cell line's viability and product expression can be increased with a growth factor. Maybe you run a cell or gene therapy process and need to reprogram or differentiate cells in your starting material. We produce GMP-grade proteins and small molecules that are designed for clinical grade manufacturing. We follow industry standards for documentation and process controls because traceability, scalability, and bioactivity are essential for ancillary materials in biologics production. Give your cells the right supplements to perform at their best. Treat them to our premium growth and production-enhancing cytokines, growth factors, and inhibitors!

PROTEINS

Each of our GMP proteins is manufactured, tested, and released under an ISO 9001:2015 and ISO 13485:2016 quality management system. You can choose from nearly 50 GMP-grade ancillary growth factors produced at our R&D Systems facility in Minneapolis. **FIGURE 8** shows a sample of the testing we do for our GMP proteins, and we have in-house capabilities for much more extensive biochemical and biophysical characterizations of that's important for your process. See the Analytical Testing Services chapter of this eBook for a description of our protein analysis platforms.

SMALL MOLECULES

GMP Small Molecules from Tocris ensure quality, safety, and security of supply for bioprocessing cell culture. Tocris® GMP Small Molecules fulfill criteria for USP <1043> classification and are manufactured, tested, and released in accordance with relevant sections of ICH Q7 guidelines. We're the exclusive source of GMP-grade small molecules!

GMP SMALL MOLECULES

- Y-27632 (ROCK inhibitor)
- SB 431542 (TGF-ß R1 inhibitor)
- CHIR 99021 (GSK-3 inhibitor)
- DAPT (γ-Secretase inhibitor)
- XAV 939 (Tankyrase inhibitor)

QC TESTING FOR GMP SMALL MOLECULES

- Purity – HPLC
- Product identification – NMR and mass spectrometry
- Water content – Karl Fischer
- Residue on ignition
- Residual solvents
- Solubility
- Bioburden
- Endotoxin

View all our GMP proteins.

Learn more about Custom Cell Culture Media.

Read more about our Quality and Documentation for GMP proteins.

DID YOU KNOW?

Running a stem cell or CAR-T cell process? See our complete resources for Cell and Gene Therapy, including our new ExCellerate™ Human T Cell Expansion Media and ExCellerate B Cell Media.

CELL CULTURE MEDIA

Robust cell culture in bioprocessing requires optimized and documented base media. Our GMP-grade, serum-free cell culture media are developed and manufactured in a controlled, traceable, and scalable process. Support your production cell line with a dependable foundation of our GMP serum-free media.

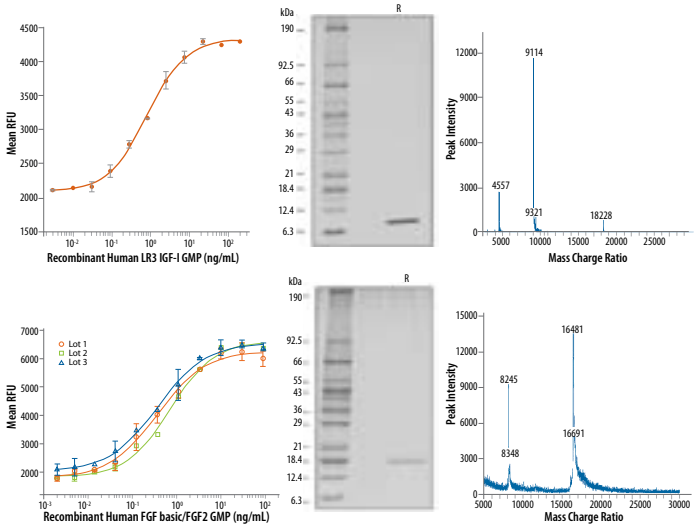
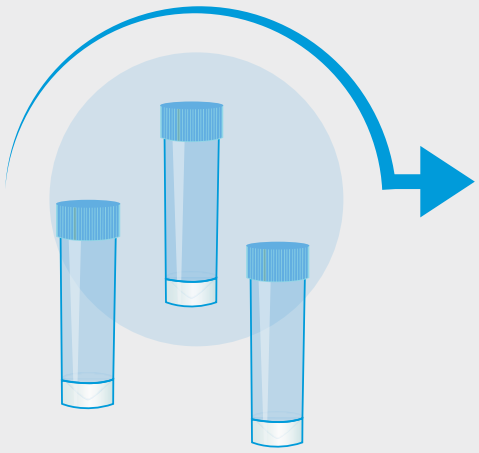


FIGURE 8 Mass spectrometry and bioassay characterization of GMP grade recombinant proteins.

IN-PROCESS CHARACTERIZATION



Keep a sharp eye on your cell culture and purification optimization; monitor both the physical and functional integrity of your product. Our unmatched instruments and services will let you run rigorous purity, identity, and activity assays to ensure that your product is what you expect.

ARTICLES IN THIS CHAPTER:

ANTIGEN SPECIFICITY AND ANTI-IDIOTYPE ANTIBODIES

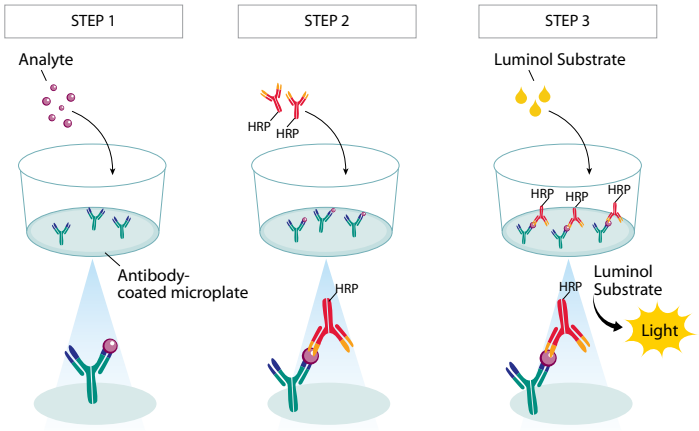
If the biologic you're producing is an antibody, it's critical to verify that it can recognize its target. Our Immunoassay Services will put world-class expertise to work for you by designing and optimizing an assay to monitor your antibody's antigen specificity. Take advantage of R&D Systems' experience from developing over 600 industry-leading Quantikine® ELISA Kits. These kits are based on premium quality antibodies and bioactive proteins, and they're exhaustively tested to ensure superior inter- and intra- assay precision, recovery, linearity, and sensitivity. Why not put that to work for you?

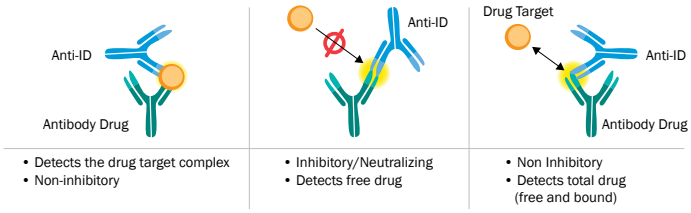
Anti-idiotype antibodies are valuable for confirming the critical epitopes of your therapeutic monoclonal. Our anti-IDs are specifically designed and tested in combination with R&D Systems® biosimilar antibodies. Our quality control testing of anti-IDs includes bridging assays, flow cytometry, binding inhibition assays, and direct ELISA (**FIGURE 9**).

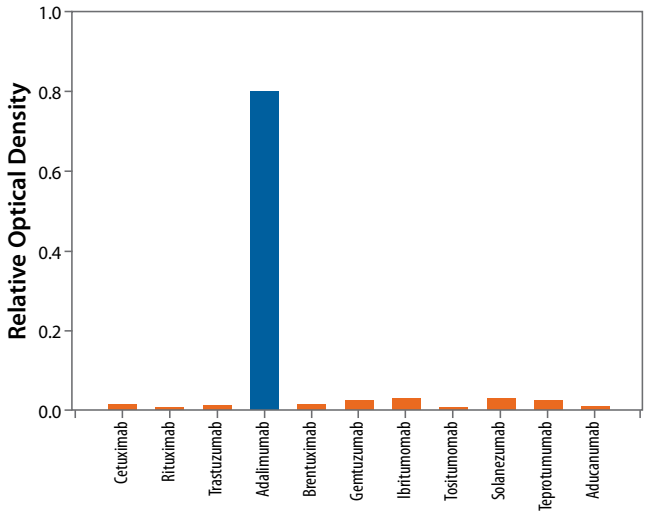
View our Anti-Idiotype Antibodies.

DID YOU KNOW?

Our Avi-tag Biotinylated Proteins are excellent for assay development. They're labeled at a single, defined lysine residue for uniform orientation on a streptavidin-coated surface.







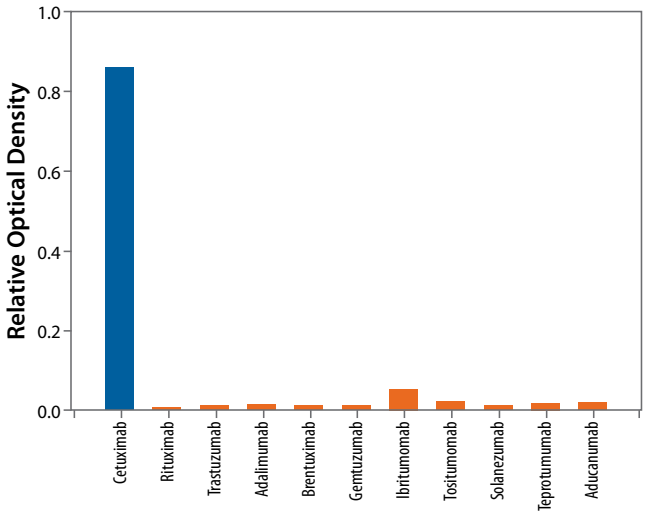


FIGURE 9: ELISA for binding specificity of Adalimumab (left) biosimilar tested with Anti-Adalimumab (Anti-Idiotype) Antibody. ELISA for binding specificity of Cetuximab (right) biosimilar tested with Anti-Cetuximab (Anti-Idiotype) Antibody.

FC RECEPTOR REAGENTS

Confirm that the constant region of your therapeutic monoclonal antibody shows the right binding or lack of binding to specific Fc receptors. Maybe you want your rmAb to bind particular FcRs and maybe you don't! We offer a complete selection of bioactive Fc Receptor proteins and FcR antibodies for use in immunoassay development (**FIGURE 10**).

View our Fc Receptor Reagents.

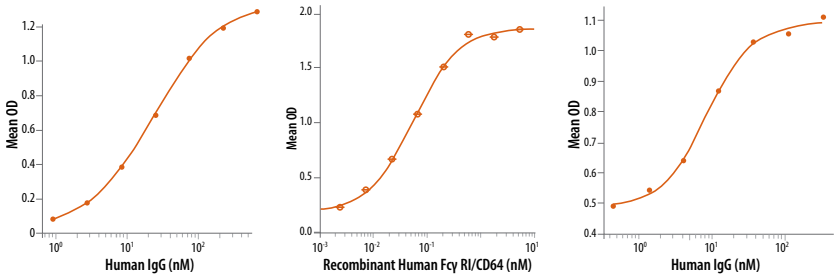


FIGURE 10: ELISA binding of recombinant Fc gamma RIIIA/CD16a, Fc gamma RI/CD64, and Fc gamma RIIA/CD32a (R167) to human IgG.

CELL-BASED BIOASSAYS

We're experts at developing and running cell-based bioassays to directly measure the bioactivity of the biologic in your sample. Our assay expertise includes proliferation, cytotoxicity, cellular differentiation, cytokine induction, chemotaxis, and much more! We've developed over 900 bioassays for the quality control of our recombinant proteins. That gives us an unmatched ability to develop and validate the best functional assay for your biologic. Our dedicated scientists can develop and run custom bioassays for you.

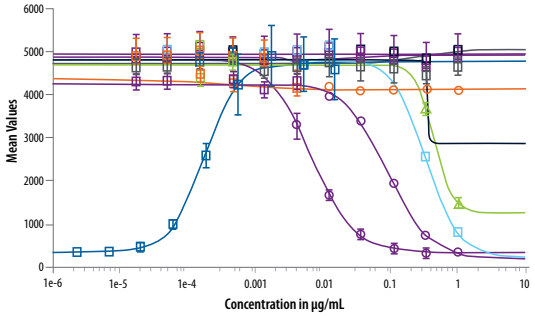


FIGURE 11: TSLP-neutralizing compounds inhibit IL-7R alpha mediated cell proliferation.

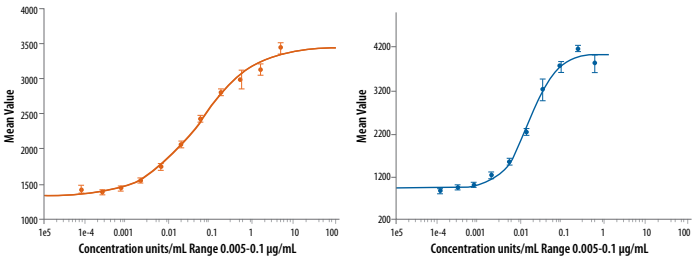


FIGURE 13: Bioactivity of Erythropoietin (left) and Growth Hormone (right).

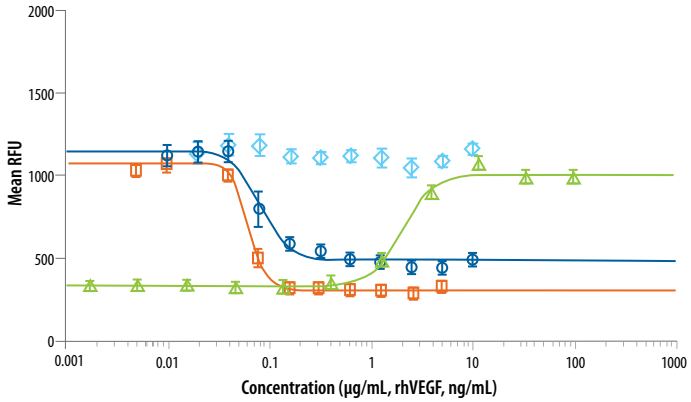


FIGURE 12: Anti-VEGF antibody neutralizes VEGF induced cell proliferation.

Learn more about about our Bioactivity Testing Services.

Explore our Bioprocessing – In-Process Characterization resources.

ENZYMATIC ASSAYS

If the biologic you're producing has an enzymatic activity, you need to verify that it's intact. Producing a coagulation factor? A coagulation inhibitor? We test the activity of every one of our recombinant enzymes with a validated assay which we can adapt to confirm the activity of your biologic enzyme. Our enzymatic assays cover proteases and their inhibitors, kinases and phosphatases, glycosyltransferases, lipid metabolism enzymes, oxidases and oxygenases, and many more activities.

View our complete Enzymes listing to see the whole range

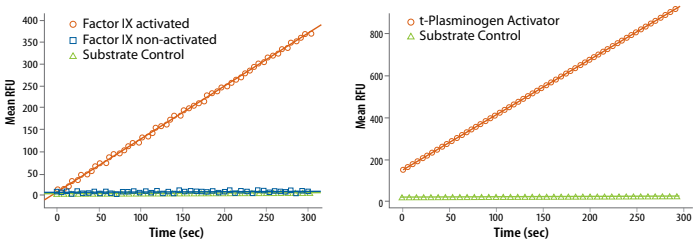


FIGURE 14: Proteolytic activity of Coagulation Factor IX (left) and t-Plasminogen Activator (right).

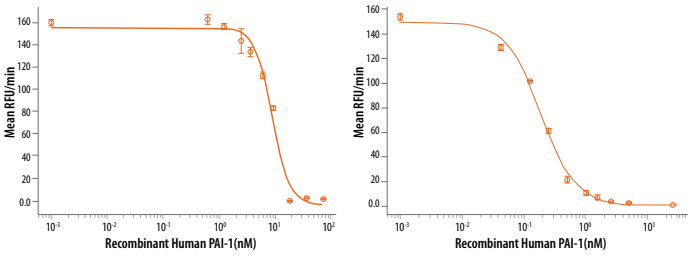


FIGURE 15: Inhibition of proteolysis by Serpin E1/PAI (left) and TFPI (right).

BIOPROCESSING ENZYMES

We offer an outstanding collection of bioactive recombinant enzymes and inhibitors. Many of these are valuable in the bioprocessing workflow, from nucleases for cleaning up your cell culture harvest material to enzymes to treat your product for purification or analysis.

Explore our Bioprocessing – In-Process Characterization resources.

DNA DIGESTION

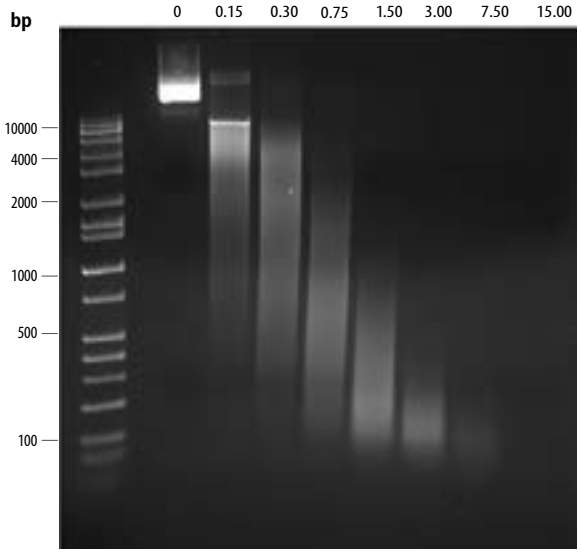


FIGURE 16: Digestion of DNA with increasing amounts of Recombinant S. *marcescens* NucA (Benzonase).

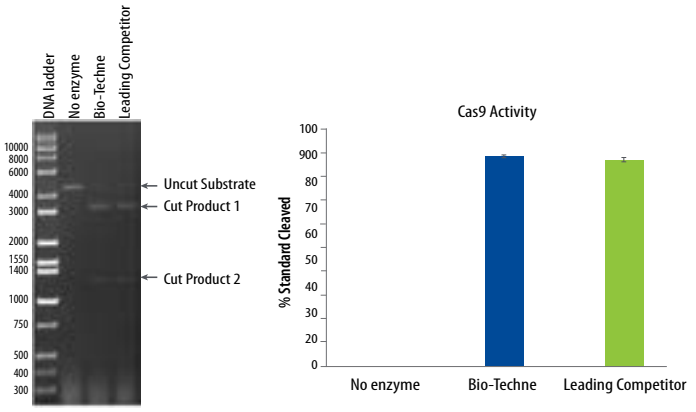


FIGURE 17: Cleavage of DNA with Recombinant S. *pyogenes* CRISPR-associated Protein 9 and a leading competitor's Cas9 (left). Quantitation of DNA cleavage by R&D Systems® Cas9 compared to a leading competitor's Cas9 (right).

BIOPROCESSING ENZYMES (CONTINUED)

GLYCOBIOLOGY-RELATED ENZYMES

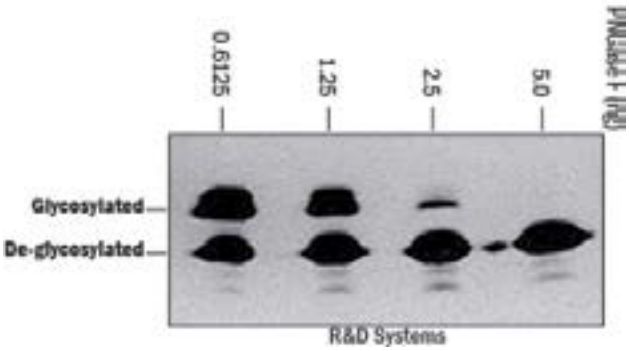


FIGURE 19: Recombinant F. *meningosepticum* PNGase F deglycosylation of RNase B at 37 °C.

HEPARINASE I, II, AND III

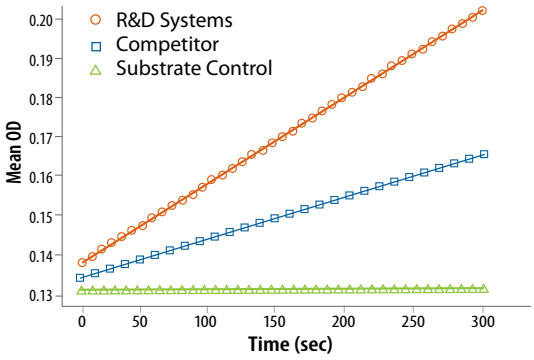


FIGURE 19: Digestion of heparin sulfate: Recombinant F.heparinum Heparinase I (orange) exhibits greater activity than a competitor's Bacteroides Heparinase I (green).

CHRONDROITINASE A, B, AC, AND ABC

CUSTOM REAGENTS & SERVICES

CAPABILITIES

- Reagents and cell culture media
- Immunoassays and bioassays
- Bulk quantity agreements and custom formulations
- Production capacity and supply chain reliability

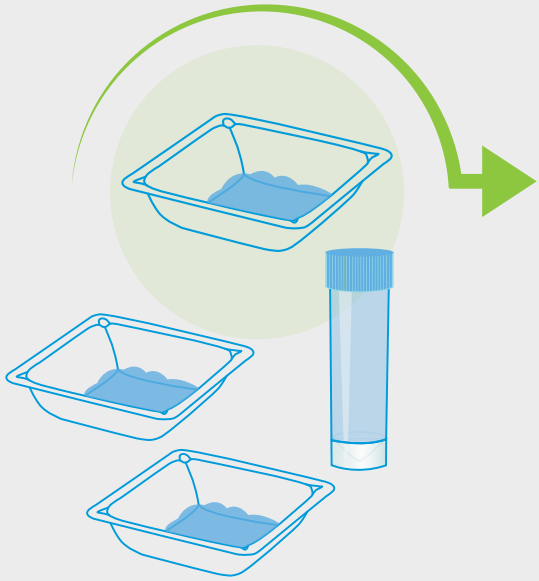
EXPERIENCE AND RELIABILITY

- Dedicated product managers and expert scientists
- Project consultations
- Documented milestones
- Communication throughout project
- Delivery of comprehensive reports

FOR MORE INFORMATION:

- Custom reagents & contract services
- Custom cell culture media
- Manufacturing and services
- Custom chemistry services
- Bioactivity testing services
- GMP-grade proteins
- Custom antibody services
- Bulk quote requests
- Custom protein services

FORMULATION



Ensure that your product is stable in solution and sails through stability stress testing. Our high-throughput instruments will help you quickly identify the optimum salts, pH, and excipients for your buffers. High throughput analytical instrumentation efficiently compares the formulation effects of multiple buffer conditions on protein aggregation, degradation, and fragmentation. Each of these platforms operates in a bioprocessing environment with 21 CFR Part 11- compliant software, so you can be confident in the integrity of your results.

ARTICLES IN THIS CHAPTER:

OPTIMIZE YOUR FORMULATION FASTER

Which buffer's best? Finding the conditions that keep your protein stable isn't exactly simple, but our instrument solutions help you narrow it all down faster. Maurice™ and iCE3™ let you run up to three times the samples in the same time it takes on other platforms, and results are ready in 15 minutes.

The number of biologics in the biopharmaceutical industry pipeline is increasing rapidly. This means that formulation process development groups carrying out product stability optimization demand high-throughput, high-precision analytical systems. Selection of the final product formulation requires subjecting the therapeutic molecule to various combinations of buffers, pH, and salt concentrations. These multivariate parameter studies routinely generate hundreds of samples needing immediate analysis, which presents throughput challenges.[1] Ion exchange chromatography (IEX) is often used for stability analysis but is usually lower in throughput than needed, with run times typically in the 30 to 60-minute range.

Scientists at Pfizer Global Biologics evaluated iCE technology during high-throughput formulation development for determining pI and separating charge-related species. They compared the stability profiles of the non-glycosylated Genotropin® with both iCE and anion exchange chromatography. The authors stated that throughput with iCE is typically higher than with ion exchange high-performance liquid chromatography (HPLC). Analysis of deamidated Genotropin® species by ion exchange HPLC took 56 minutes per sample, whereas analysis with iCE took 15 minutes or less. iCE also provides a critical advantage by eliminating the 20 to 30-minute mobilization step needed after initial focusing with traditional two-step cIEF. Elimination of the mobilization step can easily double sample throughput and also improve reproducibility.[2] Doubling the sample throughput cuts analysis time in half, a significant savings when at least 75 samples are typically analyzed at each time point.

Richard Rustandi and his colleagues at Merck developed an iCE method to determine sample pI and monitor the profiles of HBsAG VLP particles. HbsAg is a major hepatitis B virus surface antigen that self-assembles into noninfectious virus-like particles (VLP) in the vaccine.[3] The team applied their iCE method to characterize the stability of the HBsAg VLP reference standard in three different formulation buffers after repeated freeze thaw cycles. **FIGURE 20** shows the ability of iCE to identify optimum buffer conditions by variation in peak shape and resolution of species with distinct pI. Irreproducible sharp peaks observed in the electropherograms for formulations B and C are typically a sign of particle aggregation. Formulation buffer A provided the best stability for HBsAgs.[3] These iCE profiles can be used as biochemical fingerprints to demonstrate process consistency.[3] The authors stated that two important advantages of using iCE are rapid method development and high reproducibility. They added that iCE is automated, simple to run, provides a faster analysis time, and is much more robust than traditional IEF slab gel methods.

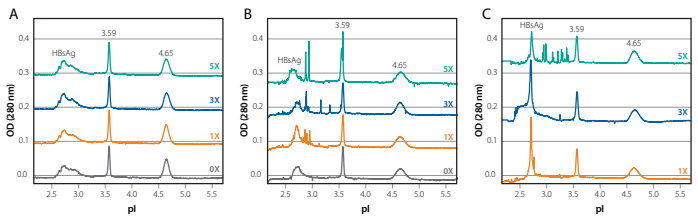


FIGURE 20: iCE electropherograms for HBsAg VLP reference standard materials. A = (formulation buffer A after 0X, 1X, 3X, 5X freeze thaw cycles), B = (formulation buffer B after 0X, 1X, 3X, 5X freeze thaw cycles), and C = (formulation buffer C after 1X, 3, 5X freeze thaw cycles).

MEET MAURICE

MEET ICE3

DID YOU KNOW?

We offer custom method development on Simple Western™ and Maurice™.

REFERENCES

1. Charge heterogeneity of monoclonal antibodies by multiplexed imaged capillary isoelectric focusing immunoassay with chemiluminescence detection, DA Michels *et al.*, *Anal Chem*, 2012; 84(12):5380–6.

2. Evaluation of the iCE280 Analyzer as a potential high-throughput tool for formulation development, N Li *et al.*, *J Pharm Biomed Anal*, 2007; 43(3):963–72.

3. Development of imaged capillary isoelectric focusing (icIEF) method and use of capillary zone electrophoresis (CZE) in hepatitis B vaccine (RECOMBIVAX HB®), RR Rustandi *et al.*, *Electrophoresis*, 2014; 35(7):1072–8.

NO-PREP, HIGH-THROUGHPUT ANALYSIS FOR HIGH SALT AND LOW CONCENTRATIONS

Chemiluminescent Simple Western™ charge assays are up to one thousand times more sensitive than absorbance-based charge heterogeneity methods and don't need extra prep for high salt or low concentration samples. Add Peggy Sue into the mix and you can analyze 96 samples in 11 hours – twice as fast as other cIEF techniques.

An important requirement in formulation development is the ability of analytical assays to detect changes in samples exposed to various buffer components and conditions. Samples containing high salt, low concentrations of protein, or both typically require desalting and sample concentration procedures prior to analysis. This not only reduces throughput and increases time to results, but also can cause unwanted sample protein alterations. Increased assay sensitivity can eliminate the need for sample cleanup and improve the pace of analysis for challenging formulation samples.

Our scientists teamed up with Genentech scientists to use Simple Western charge assays for defining the relative charge variant patterns of recombinant monoclonal antibodies (rmAbs). In one formulation study, 96 samples were tested to investigate protein concentration, buffer composition, and pH, as well as additional salt concentrations. The samples were analyzed in approximately 8 hours (5 minutes per sample) using a modified separation matrix. This is a three-fold improvement over the typical UV-based assay. Using the standard Simple Western separation matrix, similar results can be achieved in 12 hours (8 minutes per sample) for an almost two-fold improvement in throughput. As shown in **FIGURE 21**, Simple Western charge assays show a significant effect of pH on the charge distribution and can be used to support formulation development. The high specificity assay was capable of detecting product impurities even in the presence of host cell protein lysate, eliminating the requirement for sample cleanup before analysis. The authors stated that the higher specificity, speed, and sensitivity over conventional cIEF platforms qualify Simple Western technology for high throughput characterization of charge variants during rmAb formulation development.

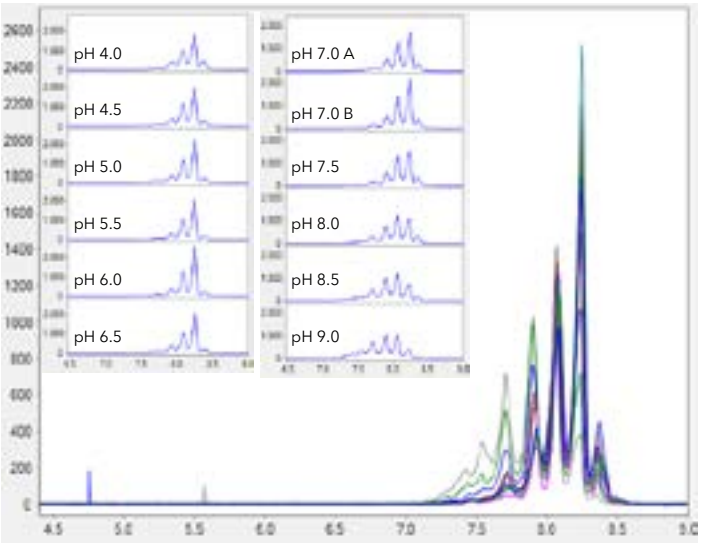


FIGURE 21: High-throughput screening of rmAb protein charge variants during formulation development using Simple Western charge assays. One of six cycles is shown comparing stability of samples formulated in buffer pH 4.0–9.0. Buffer pH 7.0 compares low salt (A) to high salt (B) concentrations. One full 96-well plate was analyzed in one run.

MEET PEGGY SUE

MEET JESS

Learn more about Simple Western™.

Looking for pricing? Request a quote.

DID YOU KNOW?

If you just need a little more sensitivity to skip desalting or concentrating your samples, try Maurice™. His fluorescence mode will give you 3–5X more sensitivity than Maurice absorbance mode and might just do the trick.

REFERENCES

1. Charge heterogeneity of monoclonal antibodies by multiplexed imaged capillary isoelectric focusing immunoassay with chemiluminescence detection, DA Michels *et al*., *Anal Chem*, 2012; 84(12):5380–6.

MULTIPLE MOLECULES, ONE METHOD

Platform methods for charge heterogeneity analysis make a huge difference in how fast you get your results. With Maurice™ and iCE3™ you can develop a method in a single day and then use it as a platform method for multiple processes. That means you can focus on what matters – testing your formulations – rather than optimizing methods for every molecule that's coming down your pipeline.

Developing platform methods is an efficient way to meet the demand of the rapidly increasing number of therapeutic recombinant monoclonal antibodies (rmAb).[1] Platform charge-based assays provide significant resource and time savings by letting you avoid having to optimize method parameters for each product you manage. Platform methods also allow you to stick with a method you're familiar with for multiple products, minimizing chances of error. Ion exchange chromatography (IEX) is often used for stability analysis, but it's not a good option as a platform method, because protocols often have to be customized to fit particular products.[2]

Xiaoping He and her colleagues at Pfizer and Genentech developed an analytical method using iCE technology for charge variant analysis and then further optimized it for use as a platform method for several IgG2 rmAbs. Using this platform iCE method, they evaluated more than 20 therapeutic rmAbs for charge heterogeneity, covering a wide range of charge compositions (acidic species ranging from 10% to 70%) and a wide pI range (6.9–9.6). The method separated rmAb charge variants with nearly baseline resolution, confirming that the method was suitable for the rmAbs tested. Charge variant profiles for a subset of the rmAbs in the evaluation are shown in **FIGURE 22**.[2] Results from this study demonstrate the method's suitability for analyzing the relative abundance of charge variants and determining pI values of charge species.[2]

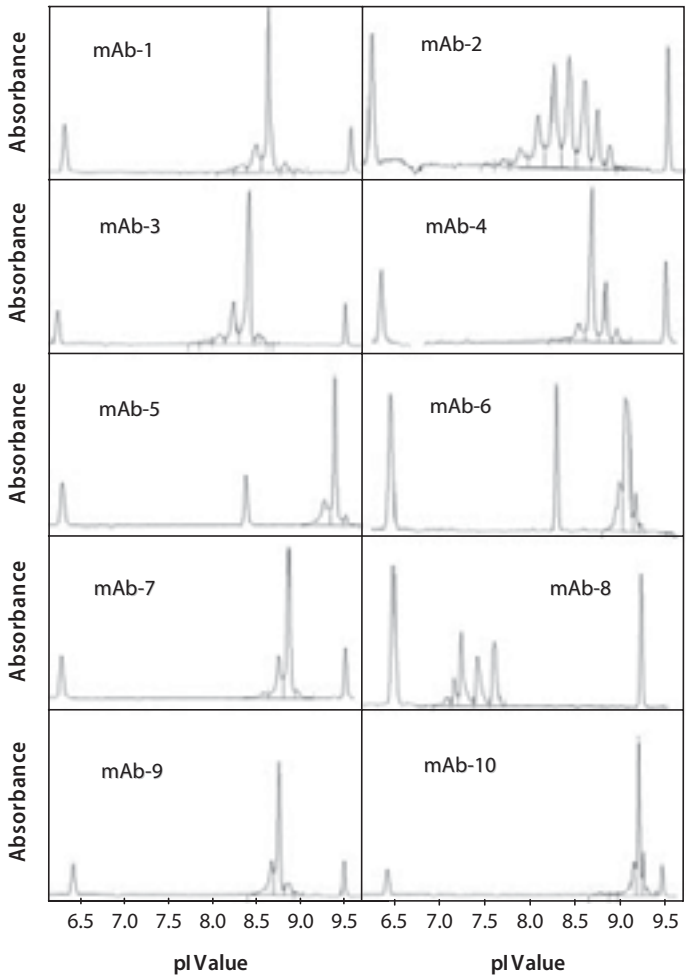


FIGURE 22: Charge variants profiles for a subset of 20 mAbs evaluated using an iCE platform method.

WATCH MAURICE VIDEO

MEET ICE3

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If you just need a little more sensitivity to skip desalting or concentrating your samples, try Maurice™. His fluorescence mode will give you 3–5X more sensitivity than Maurice absorbance mode and might just do the trick.

REFERENCES

1. Charge heterogeneity of monoclonal antibodies by multiplexed imaged capillary isoelectric focusing immunoassay with chemiluminescence detection, DA Michels *et al*., *Anal Chem*, 2012; 84(12):5380–6.

2. Analysis of charge heterogeneities in mAbs using imaged CE, XZ He *et al*., *Electrophoresis*, 2009; 30(5):714–22.

DETECT AGGREGATION EARLIER

With the MFI™ 5000 Series, you can detect those 1 µm to 100 µm aggregates that size- exclusion chromatography (SEC) has a hard time with. Detecting insoluble aggregates in the sub-visible range gives you early insight plus a better quantitative picture of your therapeutic's stability.

Optimizing buffer formulations to prevent aggregation is critical to ensure that safe and effective therapies make it to market. Protein-based therapeutics are susceptible to aggregation and particle formation which can heighten immunogenicity in vivo.[1] Aggregation of proteins in solution occurs as a result of elevated temperatures, agitation, light exposure, contact with surfaces, and freeze-thawing. Size exclusion chromatography (SEC) is often used for analysis of aggregates but is only effective for soluble aggregates in the range of 5 kDa to 1000 kDa. With SEC, insoluble aggregate formation can be indirectly measured as a loss in the amount of soluble material following treatment. For soluble aggregates, SEC sensitivity can only reliably detect changes of approximately 0.1% to 0.5% (loss of native protein and/or increase in the level of aggregates).[2] Increased sensitivity in quantifying protein aggregation allows for screening of formulations and stress conditions that are milder than those used for accelerated degradation studies.

James Barnard at the University of Colorado Department of Pharmaceutical Sciences and his colleagues at the University and at Pfizer evaluated Micro-Flow Imaging (MFI) to detect and quantify subvisible particles as a surrogate for the level of aggregated protein. Solutions of an IgG2 recombinant monoclonal antibody (rmAb) were subjected to three freeze-thaw cycles and analyzed by MFI and SEC after each freeze-thaw cycle. The authors found that even in formulations providing substantial stabilization for the rmAb, freeze-thawing resulted in the formation of thousands of particles per milliliter after the first cycle and increased particle number with two additional cycles (**FIGURE 23**), as detected by MFI. The majority of particles formed during freeze-thawing were less than 5 µm in diameter, and more than 95% of the total particles detected were less than 10 µm. MFI allowed the detection of protein aggregates comprising only hundredths of a percent of the total protein mass. Furthermore, differences in protein aggregation levels due to formulation or freeze-thaw protocol changes were resolved, even when protein aggregation could not be detected by SEC. The authors concluded that quantifying the formation of subvisible protein aggregates with MFI is far more sensitive than SEC.

MEET MFI

Looking for pricing? Request a quote.

Explore our Bioprocessing - Formulation resources.

REFERENCES

1. Structural Characterization of IgG1 mAb Aggregates and Particles Generated under Various Stress Conditions, SN Telikepalli *et al*., *J Pharm Sci,* 2014; 103(3): 796–809.
2. Subvisible particle counting provides a sensitive method of detecting and quantifying aggregation of monoclonal antibody caused by freeze-thawing: Insights into the roles of particles in the protein aggregation pathway, JG Barnard *et al*., *J Pharm Sci*, 2010; 100(2):492–503.

DETECT AGGREGATION EARLIER (CONTINUED)

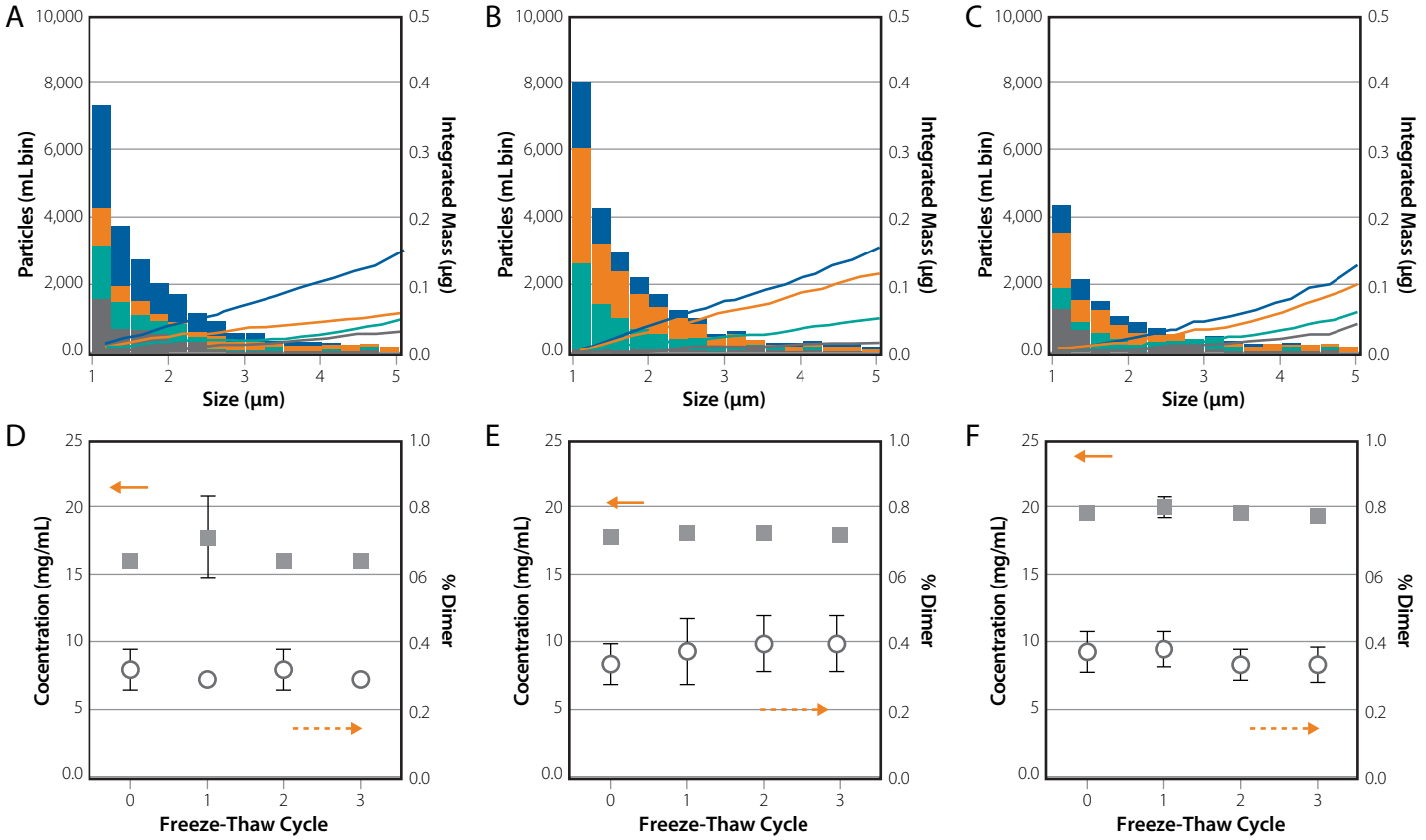

FIGURE 23: Monitoring particle formation in three different formulations using MFI and SEC. (Top) MFI particle distribution (left y-axis) and integrated mass data (right y-axis) for IgG2 samples frozen and thawed from −20 °C to room temperature. Histidine formulation with x-axis truncated at 5 µm (A); histidine PS80 formulation with x-axis truncated at 5 µm (B); and histidine, trehalose, PS80 formulation with x-axis truncated at 5 µm (C). In all panels, particle counts are for before freeze-thawing (gray), one freeze-thaw cycle (green), two freeze-thaw cycles (orange), and three freeze-thaw cycles (blue). (Bottom) Size-exclusion chromatography for histidine formulation, −196 °C to 5 °C (D); histidine, PS80 formulation, −196 °C to 5 °C (E); and histidine, trehalose, PS80 formulation, −196 °C to 5 °C (F). Closed square symbols are the soluble protein (left y-axis), and the open circles are % dimer (right y-axis). Error bars represent the standard deviation of three independent replicates. MFI data for these conditions show that despite repeated freeze/thaw cycles, SEC was unable to reliably detect changes in stability.

CLASSIFY EVERY PARTICLE, EVEN TRANSLUCENT AGGREGATES

The MFI™ 5000 Series sizes and counts subvisible particles in your samples, and it gives you morphological information too. So, you'll know exactly what particles you have in your sample – even the translucent aggregates that light obscuration (LO) misses.

Regulatory agencies require a thorough analysis of particles in a biologic, including quantitative measurements of size and count plus information on the type of the particles. Compendial methods such as LO often do not provide complete profiles due to gaps in analysis capabilities, increasing risk of failure later in development. LO suffers from an inability to discriminate between types of particles and is unable to detect translucent aggregates. Measuring protein aggregation is a common readout for product stability. The ability to measure small and potentially translucent protein aggregates provides early insight into the suitability of a formulation and the long-term stability of the product. These aggregates occur in both the USP-regulated sizes of >10 µm and >25 µm, but they're also frequently present in the 2 µm to 10 µm range described in USP 787 and have become increasingly important for biopharmaceutical products (**FIGURE 24**).

In this comparative study, MFI was found to be more sensitive than LO; it can quantitate protein aggregates that would be undetectable by LO.

REFERENCES

1. See what light obscuration misses with micro-flow imaging, ProteinSimple White Paper.

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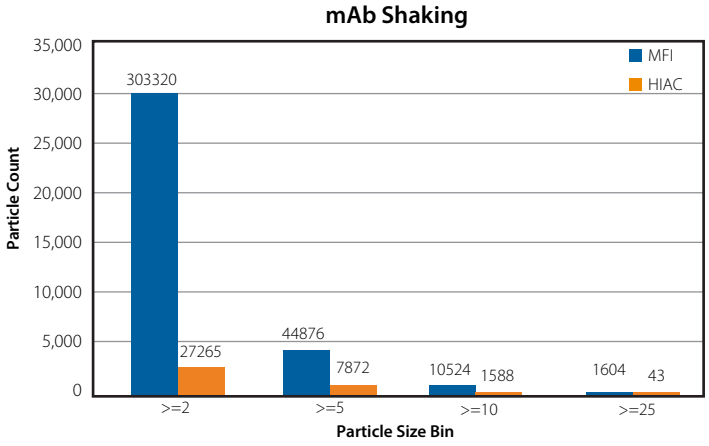

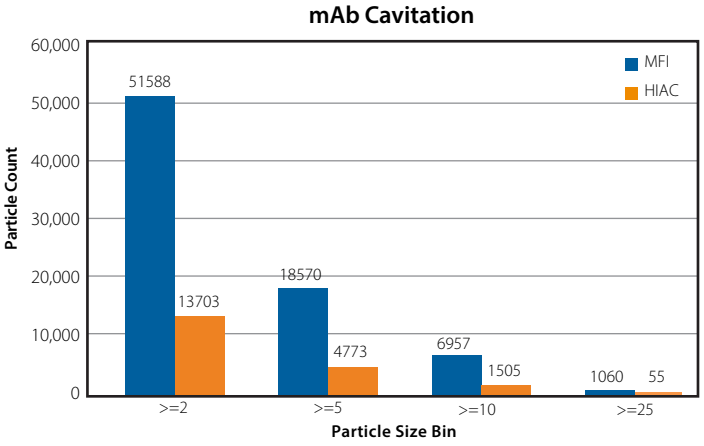

FIGURE 24: MFI detected up to 10-fold more aggregates than LO (HIAC) when comparing solutions of stressed monoclonal antibodies.

PREDICTING THE FUTURE WITH STRESS TESTS

Jess makes it easy to see what your biotherapeutic is up to during accelerated stress testing. High-resolution Simple Western™ size assays pick up specific antibody fragments. Jess also runs 25 samples in 3 hours flat, and you only need 3 µL per sample.

Rigorous evaluation of product degradation is a regulatory requirement for critical quality attribute (cQA) monitoring of biologics production. The complexity of impurity formation during accelerated stress testing of recombinant monoclonal antibodies (rmAbs) typically requires testing with multiple analytical methods. Size-exclusion chromatography (SEC) is commonly used to detect rmAb fragmentation but has limited ability to resolve low molecular weight (LMW) species. CE-SDS provides better LMW fragment resolution than SEC, but coelution of impurities can lead to underestimation of impurity content. Structural variation can impact IgG stability and complicate the characterization of IgG fragmentation. Orthogonal approaches that address these issues are needed.

Scientists at Bristol-Myers Squibb (BMS) developed a rapid characterization and quantification method for protein fragmentation analysis with the Simple Western size assay and used the assay to monitor fragmentation of heat-stressed rmAb samples. The two assays were evaluated with detection antibodies selective for Fc and F(ab) fragments of IgG_1. With these antibodies, the Simple Western size assay resolved fragments of 110 kDa and 50 kDa which were not resolved by SEC (**FIGURE 25**). The BMS team also evaluated the assays for quantification of rmAb fragments in heat stressed samples containing both IgG_1 and IgG_4 antibodies. They were able to monitor the 110 kDa fragment in both single IgG_1 samples and mixed IgG_1 plus IgG_4 samples (**FIGURE 26**). The authors concluded that Wes is a valuable orthogonal technology for the characterization and evaluation of rmAb fragments.

MEET PEGGY SUE

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REFERENCES

1. Evaluation of Automated Wes System as an Analytical and Characterization Tool to Support Monoclonal Antibody Drug Produce Development, J Wang *et al.*, *J Pharm Biomed Anal*, 2017; 139:263–268.

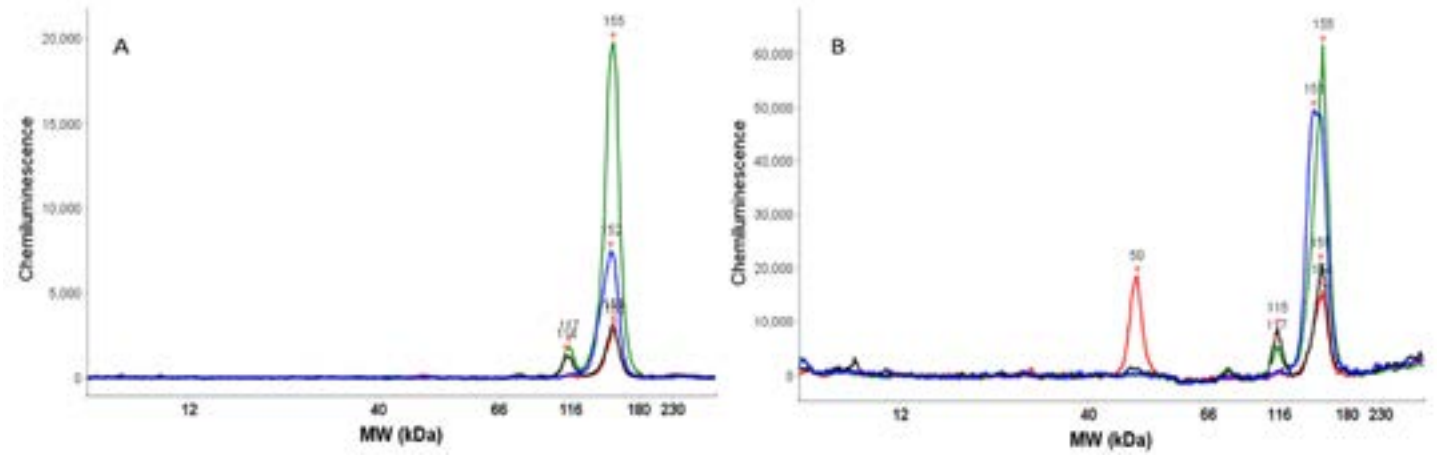

FIGURE 25: Fractions collected from SEC analyzed using the Simple Western size assay on Wes. Fraction 1 (blue), fraction 2 (black), fraction 3 (green), and fraction 4 (red) were detected (A) using Anti-IgG1(Fc)-HRP (clone 6069) and (B) using Anti-IgG (Fab)-HRP.

PREDICTING THE FUTURE WITH STRESS TESTS (CONTINUED)

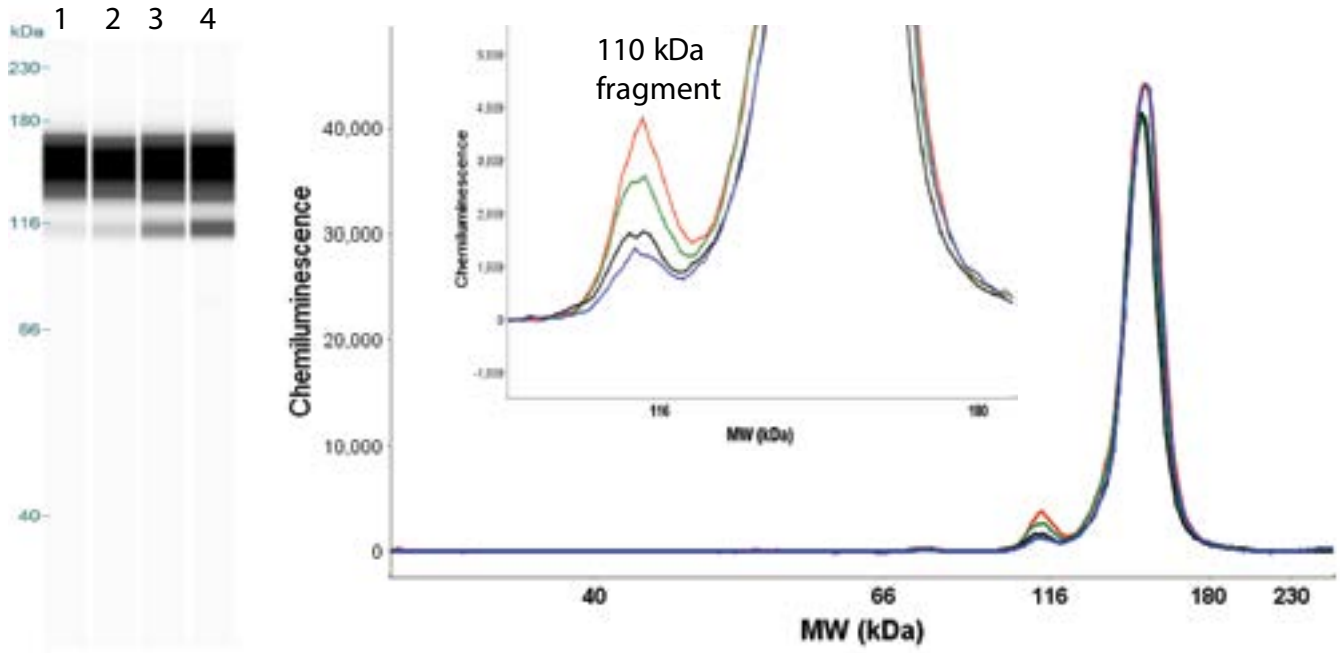

FIGURE 26: Heat stressed IgG1 and IgG4 mixture and non-stressed mixture analyzed by the Simple Western Size assay on Wes using Anti-IgG1(Fc)-HRP (clone 6069). An increase of the 110 kDa fragment from 2.19% on non-stressed mixture samples to 5.53% on a 3-month, 40 °C stressed mixture sample was observed. Based on the specificity of clone 6069 for IgG1, results suggest the 110 kDa fragment originates from IgG1 in the mixture sample. Results shown as gel-like image (left) and electropherogram (right). Lane 1/blue trace: non-stressed mixture, lane 2/black trace: 40 °C, 1-month treated mixture, lane 3/green trace: 40 °C 2-month treated mixture, lane 4/red trace: 40 °C, 3-month treated mixture.

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With just a little more sample, you'll see everything you need to with Maurice™ and Maurice S.

DID YOU KNOW?

We offer custom method development on Simple Western™ and Maurice™.

FINAL PRODUCT CHARACTERIZATION

Rely on our robust and thoroughly validated instrument platforms. They'll demonstrate the integrity of your product and confirm that residual contaminants are within regulatory limits.

ARTICLES IN THIS CHAPTER:

COVER YOUR CQAS WITH HIGH-THROUGHPUT, HIGH-RESOLUTION ANALYSIS

It goes without saying that charge heterogeneity is a critical quality attribute (CQA) for the testing required by regulatory agencies. But did you know Maurice™ and iCE3™ let you hand it over faster? You'll have results in just 10 minutes per sample and with the resolution you need to quantitate CQAs.

Characterization of the charge heterogeneity of recombinant proteins is an important part of biopharmaceutical product development. Fast, robust, quantitative, high- throughput analysis is needed to support stability testing and multiple product releases. Isoelectric focusing (IEF) slab gel and/or ion-exchange chromatography (IEX) have traditionally been used to monitor protein charge heterogeneity for batch-to-batch process consistency, product quality assessment, and characterization of protein stability and purity. IEX requires significant method development time and often does not provide adequate resolution for isoform quantitation. Slab gel IEF is laborious and isn't quantitative, and it lacks the resolution needed for basic molecules. Compared with IEX, cIEF techniques demonstrate superior resolution, shorter method development time (especially with platform methods), higher throughput, and reduced waste generation. In conventional cIEF analysis, protein isoforms are first focused along the capillary according to their isoelectric point and then mobilized past a fixed detector. The mobilization step adds 20 to 30 minutes to the run time and can affect data resolution. Whole column imaged cIEF (icIEF) technology overcomes this issue with direct, whole-column detection that eliminates the need for a mobilization step.

Scientists at Biogen developed an icIEF-based iCE method for quantitative analysis of recombinant mAb (rmAb) charge heterogeneity. A final iCE separation profile for the rmAb is shown in **FIGURE 27**. Method development work was completed within two days, a significantly shorter time than is required for developing an IEX method. A representative IEX chromatogram for the rmAb is shown. The authors stated that iCE offers faster analysis and requires less development work. A typical analysis can be done in 15 to 20 minutes compared with over 60 minutes with IEX. Additionally, small differences in mobile phase preparation or column packing with IEX may result in altered protein isoform migration times and failure to meet the assay acceptance criteria. In contrast, pI shifts with iCE are not an issue for a selected sample matrix, resulting in better profile reproducibility. The authors found that the iCE method is not only suitable and also advantageous as a release and stability assay for charge heterogeneity determination. They stated that it can be successfully used as a replacement for IEX.

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1. Application of imaging capillary IEF for characterization and quantitative analysis of recombinant protein charge heterogeneity, Z Sosic *et al.*, *Electrophoresis*, 2008; 29(21):4368–76.

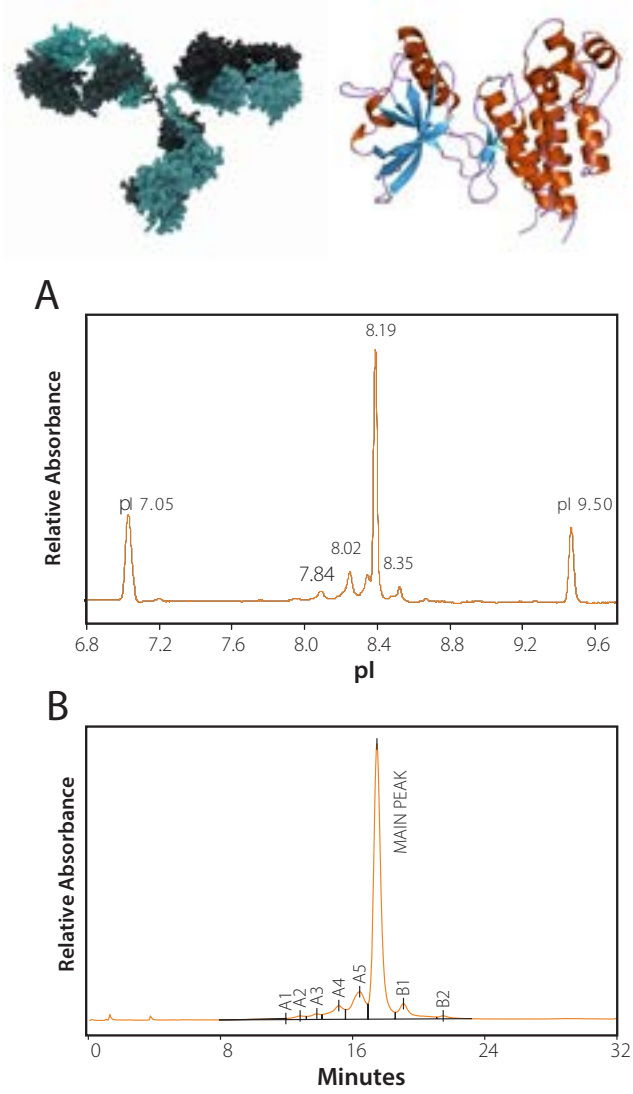

FIGURE 27: Comparing icIEF with IEX. (A) iCE profile and (B) IEX profile for rmAb A1–A5 are acidic peaks 1–5, B1–B2 are basic peaks 1 and 2. iCE resolved more charge variants compared to IEX. The acidic shoulder next to the main peak was also more resolved with iCE compared to IEX.

DID YOU KNOW?

You can't go wrong with absorbance-based assays on iCE3™ or Maurice™. But if you need more sensitivity, try Maurice's native-fluorescence mode. With 3–5X increased sensitivity and less susceptibility to ampholyte noise, it detects things absorbance can't. Plus, you can compare electrograms in absorbance and fluorescence to identify artifacts.

TACKLE DIFFICULT PROTEINS

Need to monitor ADCs or fusion proteins? Ion-exchange chromatography (IEX) doesn't quite cut it in the resolution department, but Maurice™ and iCE3™ easily resolve these not-so-easy to separate proteins and make analyzing them totally doable.

Fusion proteins with complicated charge variants or hydrophobic antibody-drug conjugates (ADC) often are incompletely resolved by ion-exchange chromatography (IEX).

Joyce Lin and Alexandru C. Lazar at Immunogen used iCE to monitor the charge heterogeneity profile of conjugated antibodies in an ADC product and quantitate the amount of unconjugated antibody. Immunoconjugates are heterogeneous, containing antibody molecules that carry different numbers of the cytotoxic agent. Drug conjugation to primary amino groups in the antibody eliminates positively charged sites and changes the antibody's pI. Traditionally, analytical methods such as IEX and isoelectric focusing (IEF) in slab gel and capillary formats are used to monitor protein charge heterogeneity and to measure isoelectric points (pI). However, the authors' preliminary experiments showed that neither IEX nor conventional IEF provided sufficient resolution to separate the charge variants in ADC products, due to the small differences in pI values between antibodies carrying different numbers of payload molecules. They also noted that iCE provides increased peak separation, because the mobilization step in conventional cIEF often leads to peak broadening, poor reproducibility, and reduced resolution. The authors generated an iCE profile using unconjugated antibody and used this profile to identify the peak of unconjugated antibody in their immunoconjugate samples (**FIGURE 28**). They determined the relative concentrations of various immunoconjugate species by comparing the areas under each peak. Determination of percent unconjugated antibody in the ADC sample was made possible by the higher resolution achieved with iCE.

REFERENCES

1. Determination of charge heterogeneity and level of unconjugated antibody by imaged cIEF, J Lin and AC Lazar, *Methods Mol Biol,* 2013; 1045:295–302.

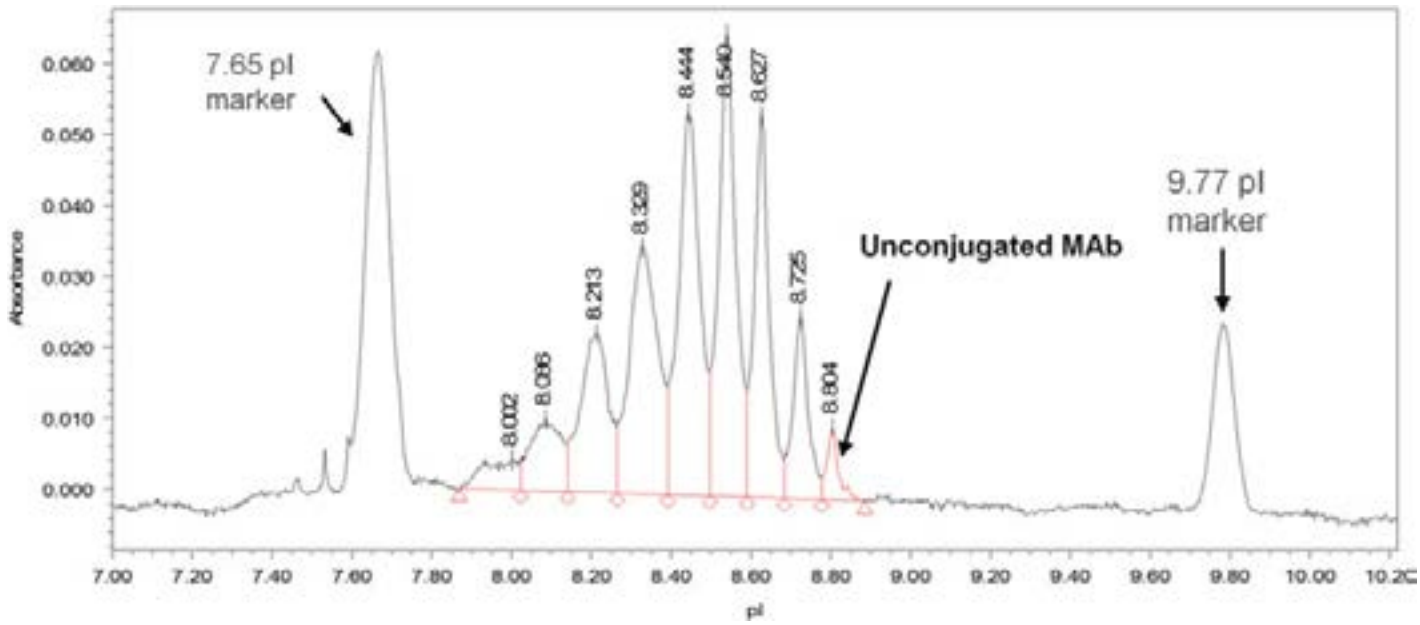

FIGURE 27: Electropherogram of immunoconjugate sample showing unconjugated antibody and conjugates with variable amounts of conjugated drug.

MAURICE EMPOWER CONTROL KIT

Maurice™ is your cIEF and CE-SDS platform that delivers high-resolution, reproducible identity, purity, and heterogeneity analysis of biotherapeutic proteins. Maurice can be controlled using Compass for iCE software, but he is also supported by Waters™ Empower® 3 Chromatography Data Software for seamless integration into your laboratory environment.

MONITORING AND VIEWING CIEF DATA

Samples were analyzed using a Maurice cIEF method and were detected using absorbance and, for higher sensitivity, native fluorescence. The Maurice cIEF System Suitability Kit was run with an IgG standard in 10 replicate injections for each batch and focused for 1 minute at 1500 V followed by 8 minutes at 3000 V. The samples were run on both a Maurice controlled by Empower and a Maurice controlled by Compass for iCE. Sample separation on the Maurice controlled by Empower was monitored in real time in the Empower "Run Samples" window. The focusing series can also be visualized spatially using a 3D spectral plot (**FIGURE 29**). This allows you to see when peaks reach optimal focusing in one image instead of comparing a series of 2D image slices taken during the separation every 10 seconds.

REPRODUCIBLE AND EQUIVALENT CIEF RESULTS

The data generated with Empower and Compass for iCE were analyzed to evaluate data reproducibility. Data consistency was demonstrated with an Empower overlay of three consecutive injections of the IgG standard and quantification of the data; CVs were all under 2.9% for 10 injections (**TABLE 1**). Quantitation also established the equivalency in Maurice data with either Empower software or Compass for iCE. The percent peak composition for peaks >10% were all within 0.5%, well within expected run-to-run and sample preparation variance requirements.

REPRODUCIBLE AND EQUIVALENT CE-SDS RESULTS

Reduced IgG standard (1 mg/mL) was run in 10 replicates on an Empower controlled Maurice and a Compass for iCE-controlled Maurice. The samples were separated for 25 minutes at 5750V, and sample separations were monitored in real time in the Empower "Run Samples" window. An overlay of three consecutive injections of the IgG standard using Empower software visually established the data reproducibility achieved with Maurice.

Quantification of the data further confirms data consistency, as all CVs were under 1.0% when samples were run via Empower or Compass for iCE. Using Empower to analyze runs controlled by either software platform demonstrates data equivalency as the percent peak areas for the reduced light chain (LC), nonglycosylated heavy chain (NGHC), and heavy chain (HC) were all within 0.3% (**TABLE 2**). All data were reproducible, with CVs less than 0.7%.

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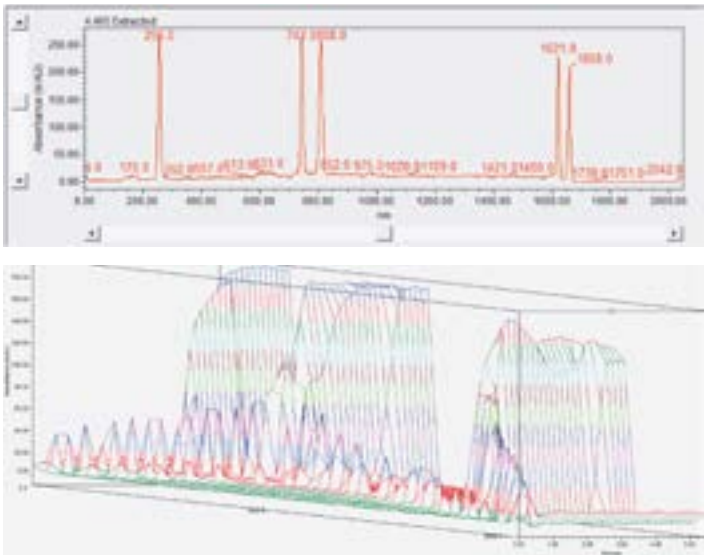

FIGURE 27: Spectrum data for the System Suitability in 2D (top) and 3D (bottom). In the 3D plot, time is on the x-axis, absorbance is on the y-axis, and pixel position is on the z-axis.

MAURICE EMPOWER CONTROL KIT (CONTINUED)

	EMPOWER® % COMPOSITION (FLUORESCENCE)			COMPASS FOR ICE % COMPOSITION (FLUORESCENCE)		
INJECTION	PEAK 2	PEAK 3	PEAK 4	PEAK 2	PEAK 3	PEAK 4
1	13.76	36.19	43.48	13.88	36.82	43.72
2	13.82	36.25	42.90	13.95	36.61	43.40
3	12.94	37.47	44.08	14.10	36.35	43.76
4	13.44	36.67	42.54	14.00	37.47	43.75
5	13.98	36.58	43.33	13.69	37.86	44.38
6	13.56	36.11	42.60	13.78	37.57	43.75
7	13.13	37.07	44.00	13.81	36.72	44.70
8	13.75	35.45	42.89	14.17	36.43	43.14
9	12.88	36.87	42.93	13.87	36.94	43.87
10	13.59	36.74	44.05	14.43	37.78	43.36
Mean	13.48	36.54	43.28	13.97	37.06	43.78
% RSD	2.83	1.55	1.38	1.57	1.52	1.06

TABLE 1: cIEF data summary of IgG standard peaks with a percent composition >10%, run on Maurice controlled either by Empower or by Compass for iCE.

	EMPOWER®			COMPASS FOR ICE		
INJECTION	LC % PEAK AREA	NGHC % PEAK AREA	HC % PEAK AREA	LC % PEAK AREA	NGHC % PEAK AREA	HC % PEAK AREA
1	24.09	8.96	58.51	24.55	8.87	57.92
2	24.05	9.00	58.53	24.40	8.88	58.09
3	24.15	8.98	58.35	24.30	8.92	58.18
4	24.14	8.93	58.44	24.36	8.90	58.22
5	24.01	8.95	58.36	24.31	8.88	58.26
6	24.07	8.99	58.48	24.31	8.87	58.29
7	24.09	8.98	58.49	24.39	8.87	58.11
8	24.05	8.97	58.45	23.97	8.92	58.66
9	23.76	8.86	59.04	24.35	8.91	58.20
10	24.08	8.99	58.54	24.24	8.88	58.33
Mean	24.05	8.96	58.52	24.32	8.89	58.23
% RSD	0.46	0.46	0.33	0.61	0.23	0.33

TABLE 2: CE-SDS data summary for the % peak area of reduced IgG standard run on either an Empower® controlled Maurice or Compass for iCE-controlled Maurice.

USER-PROOF, SITE-PROOF DATA

Maurice™ and iCE3™ take the stress out of transferring methods. Simple workflows, high resolution, and automation cut the variability to keep your data robust and consistent no matter where it's collected.

As most biotherapeutic organizations have facilities worldwide, robust analytical techniques are needed to measure the charge heterogeneity of therapeutic proteins to ensure consistent data quality between the different analysts and multiple sites. Platform methods for charge heterogeneity analysis are also highly desirable as they decrease the potential for error.

An international collaboration of 12 laboratories from 11 independent biopharmaceutical companies in the United States and Europe evaluated the precision and robustness of iCE technology for the charge heterogeneity analysis of recombinant monoclonal antibodies (rmAb). Each lab used the same iCE method to determine the apparent pI and the relative distribution of charged isoforms for a representative rmAb sample. All participants in the study used the same type of iCE system and autosampler, and the same test rmAb sample was provided to each of the 12 laboratories. Consistent electrophoretic profiles were obtained by all laboratories. The apparent pI data generated for each charged variant peak showed very good precision between laboratories with relative standard deviation (RSD) values of less than 0.8%. **TABLE 3** shows the relative distribution of the rmAb charge variants in samples prepared using the same ampholyte lot, indicating strong data robustness across different sites and different users. RSDs across sites for major peaks (percent peak area > 10%) were within 3.9%, and RSDs for all peaks were within 3%. **TABLE 4** shows the precision results for the iCE charge heterogeneity analysis of the representative rmAb samples across different laboratories, different analysts, different lots of ampholytes, and multiple instruments. The authors stated that there is strong correlation between the percent peak area values of the charge variants for antibody samples prepared using ampholyte lot A1 and lot A2. This inter-laboratory study highlighted that iCE is a robust technology for determining the charge heterogeneity of therapeutic antibodies and for use as an alternative or complementary methodology to conventional cIEF technology. The results encourage the use of iCE in support of process development and regulatory submissions of therapeutic antibodies

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1. Robustness of iCIEF methodology for the analysis of monoclonal antibodies: An interlaboratory study, O Salas-Solano *et al*., *J Sep Sci*, 2012; 35(22):3124-9.

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	COMPANY AVERAGE PERCENT PEAK AREA						
	PEAK 1	PEAK 2	PEAK 3	PEAK 4	PEAK 5	PEAK 6	PEAK 7
A	2.09	9.49	22.6	31.5	24.3	8.97	1.12
B	1.87	9.36	22.5	31.7	24.2	9.08	1.31
C	2.15	10.2	23.4	30.9	23.7	8.47	1.13
E	1.92	10.1	22.7	31.3	23.9	8.88	1.22
H	2.14	8.81	21.8	31.4	24.6	9.81	1.43
I	3.00	11.8	23.6	29.7	22.0	8.36	1.57
J	1.85	9.31	22.6	31.3	24.8	9.07	1.16

TABLE 3: Average percent peak area of the rMAb-charged variants across organizations (ampholyte A2). The values represent, for each organization, the average values of 12 iCE runs from two different samplepreparations injected six times each.

USER-PROOF, SITE-PROOF DATA (CONTINUED)

	AMPHOLYTE LOT A1						AMPHOLYTE LOT A2					
	ALL DATA			WITHOUT OUTLIERS			ALL DATA			WITHOUT OUTLIERS		
	MEAN A	SD	% RSD	MEAN B	SD	% RSD	MEAN C	SD	% RSD	MEAND	SD	% RSD
Peak 1	2.08	0.36	17	2.20	0.18	8.2	2.15	0.37	18	2.10	0.13	6.2
Peak 2	9.88	0.30	3.1	9.61	0.30	3.0	9.88	0.30	3.1	9.88	0.30	3.1
Peak 3	23.0	0.73	3.2	23.1	0.70	3.0	22.7	0.56	2.5	22.7	0.56	2.5
Peak 4	31.1	0.45	1.5	31.1	0.45	1.5	31.1	0.63	2.0	31.0	0.66	2.0
Peak 5	23.9	0.75	3.1	23.9	0.72	3.1	23.9	0.86	3.6	24.1	0.38	1.6
Peak 6	8.74	0.55	6.3	8.85	0.45	5.3	8.95	0.44	4.9	8.95	0.44	4.9
Peak 7	1.25	0.22	18	1.30	0.14	11	1.28	0.16	12	1.23	0.11	9.0

TABLE 4: Precision results for the relative distribution of the rMAb-charged variants. a) n = 144 data points for each peak. b) n = 144 data points for peaks 4 and 5; n = 120 data points for peaks 1, 2, 3, 6 and 7. c) n = 84 data points for each peak. d) n = 84 data points for peaks 2, 3, 4 and 6; n = 72 data points for peaks 1, 5 and 7.

A FASTER, SIMPLER CE-SDS TECHNIQUE FOR PRODUCT IDENTITY

CE-SDS on Maurice™ isn't the usual tedious and complicated routine. All it takes is a simple, 10-minute setup. Every step of the assay happens in a ready-to-go cartridge, and Maurice automates the whole process on up to 100 samples per batch. He gives you protein purity plus site occupancy and also detects low abundance clips with a baseline that's more likely to stay put—so your quantitation's better too.

CE-SDS is widely used for size heterogeneity monitoring of both product- and process- related impurities. A CE-SDS profile provides a fingerprint of the size variants produced during product manufacturing including combinations of light and heavy chain antibody fragments, non-specific cleavage products, and non-dissociable HMW aggregates. It's also useful for tracking host cell proteins and contamination by materials outside of the manufacuring stream.1 However, the traditional CE-SDS systems used for this analysis can suffer from drifting baselines which affect quantitative results. In addition, tedious workflows limit unattended throughput, and complicated analysis procedures require the direct involvement of experts.

Our scientists performed a comprehensive assessment of Maurice's CE-SDS performance and evaluated its comparability to other CE instrumentation. A NIST monoclonal antibody (NISTmAb) reference material was used for analysis, as it is compositionally similar to other therapeutic mAbs. Reduced and non-reduced CE-SDS separations were run on Maurice and the SCIEX PA 800 and then compared with results obtained on the PA 800 Plus* published by NIST. For the reduced NISTmAb, all three systems baseline resolved the non-glycosylated heavy chain (NGHC) and the heavy chain (HC) and detected very small NGHC and thioester peaks (**FIGURE 30**).

Percent peak areas across all three systems were within 4.6% for the major peaks and within 0.2% for the minor species. Relative abundance for each peak on Maurice was very precise with CVs ≤ 0.3% for peaks at greater than 10% relative abundance. The NGHC and thioether relative peaks of <1% abundance had CVs

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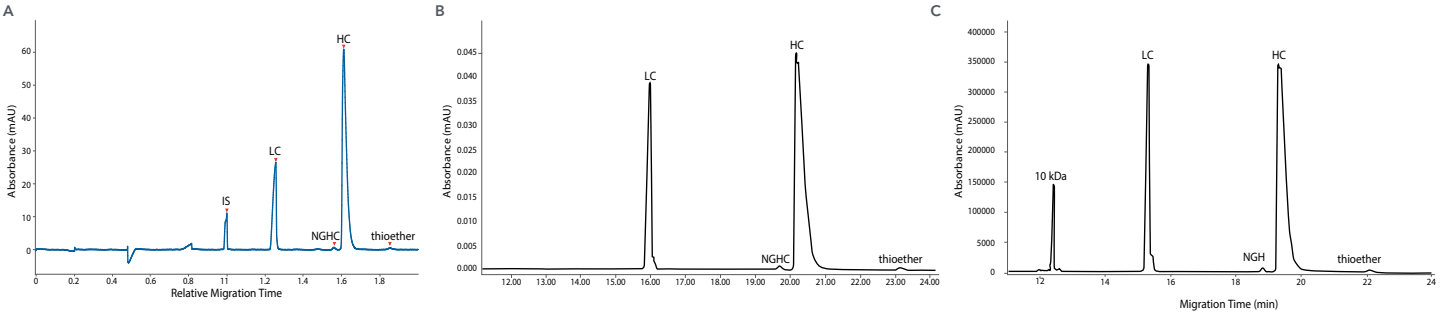

FIGURE 30: Maurice (A), the PA 800 (B), and the PA 800 Plus (C, run by NIST4) each baseline-resolved the 1 mg/mL NISTmAb (RM 8971). All systems resolved and detected the light chain (LC), non-glycosylated heavy chain (NGHC or NGH), heavy chain (HC), and thioether peak. Visually, the peak height of the light chain (LC) in the PA 800 and PA 800 Plus was higher with a smaller full width, half max compared to Maurice, but quantitation of peak areas showed overall areas were comparable.

A FASTER, SIMPLER CE-SDS TECHNIQUE FOR PRODUCT IDENTITY (CONTINUED)

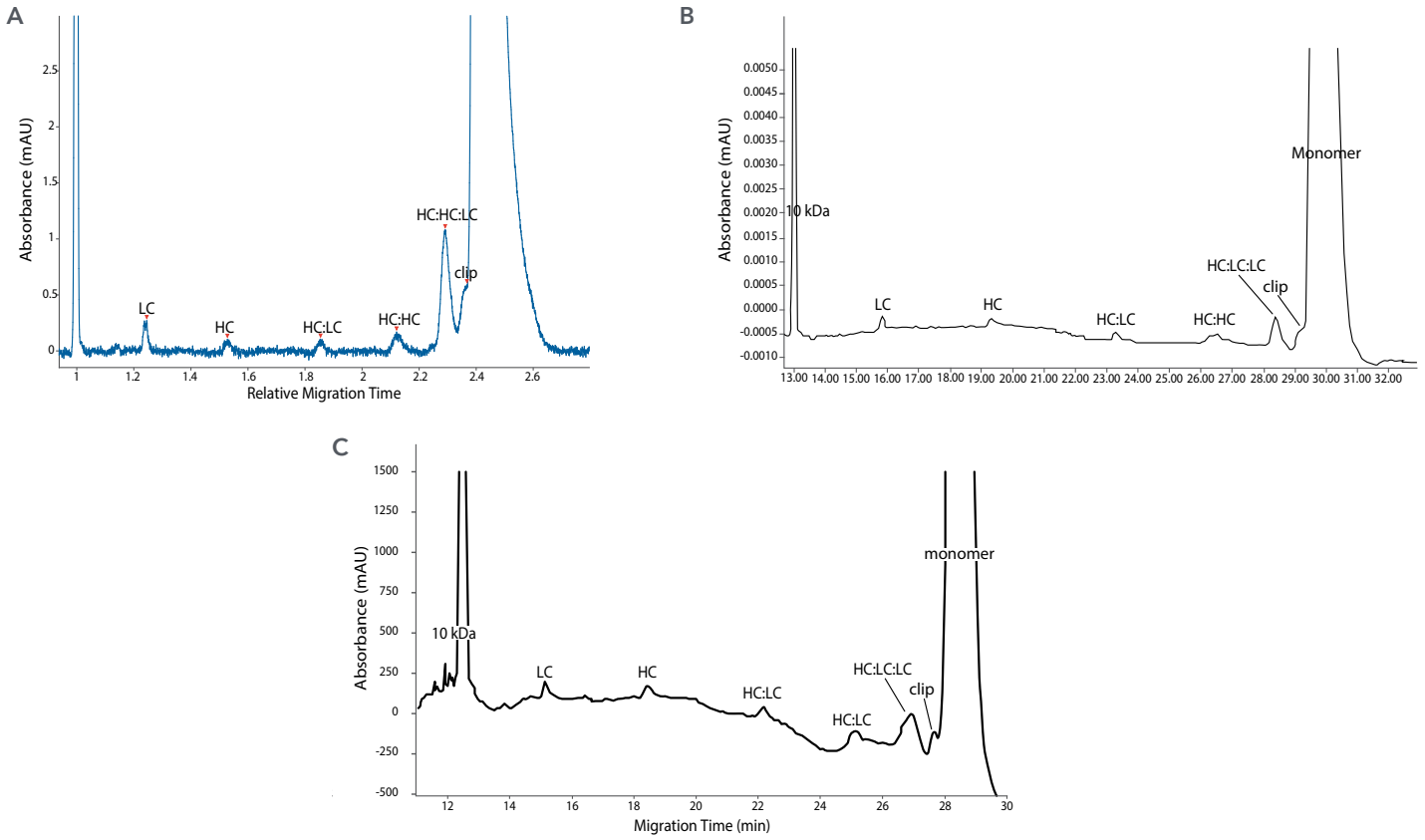

FIGURE 31: Maurice (A), the PA 800 (B) and the PA 800 Plus (C, run by NIST4) all detected five low molecular weight antibody fragments (assumed to be the Light, Heavy, Heavy-Light, Heavy-Heavy and Heavy-Heavy-Light fragments), one unidentified clip and the intact monomer in 1 mg/mL of non-reduced NISTmAb (RM 8671). Peak patterns between the systems are very similar though there is significantly less baseline drift in the Maurice, especially in the region where higher molecular weight aggregates are typically detected.

≤4.7%, demonstrating system precision for minor peaks. The PA 800 provided comparable quantitation with slightly higher CVs for the lower abundance peaks.2 Analysis of the nonreduced NISTmAb (**FIGURE 31**) on all instruments showed CVs of 0.2%. Maurice provided higher data reproducibility than the PA 800 for the LMW species (CVs of 9.0% and 12.0%, respectively). In addition to providing improved data quality for size determination of denatured samples, Maurice streamlines the application workflow. Required setup time is approximately 10 minutes. All assay steps are performed in a reusable cartridge and are fully automated, minimizing potential for error and increasing data quality. Maurice's automation also enables scientists to analyze up to 96 samples unattended which allows for increased laboratory throughput.

*Quantitative data not available for PA 800 Plus, data generated externally by NIST.

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2. CE-SDS analysis of a NISTmAb reference standard using both Maurice and the SCIEX PA 800/PA 800 Plus, ProteinSimple Application Note.

3. National Institute Report of Investigation for Reference Material 8671, Lot # 14HB-D-002.

HIGH-THROUGHPUT VACCINE SEROTYPE IDENTIFICATION

Did you know Simple Western™ assays can act like pseudo dot blots? You'll get the sensitivity and high specificity you need for the WHO, and Peggy Sue turns 96 samples around in 24 hours. That's two to three times faster than ELISA and manual dot blot Western without the high failure rates. Can't beat that!

Streptococcus pneumonia bacterial infections are responsible for the majority of mortality and morbidity seen in very young infants, the elderly, or the immune compromised population. The World Health Organization (WHO) recommends that an identity test be performed for pneumococcal conjugate vaccines in both the monovalent bulk product and the final container product containing all of the intended polysaccharide serotypes plus carrier protein. The identity test should be highly specific and based on unique aspects of the particular polysaccharide and protein structure, but it is typically qualitative. Nuclear magnetic resonance (NMR) spectroscopy is the gold standard for identity testing of non-conjugated polysaccharides. However, this method does not work for conjugate polysaccharides due to the presence of a protein carrier. Traditional immunoassays, such as ELISA and/or a manual dot blot Western, are employed as identity tests for polysaccharide conjugate vaccine products, but both techniques are usually time consuming and labor intensive. In addition, both methods are problematic for multivalent vaccines and can be difficult to transfer to other laboratories. Therefore, a faster and more automated method is highly desirable.

Melissa Hamm and her colleagues at Merck Research Laboratories developed an automated, qualitative, Simple Western identity assay for a 15-serotype pneumococcal conjugate vaccine. Simple Western size assay conditions were optimized to allow the large vaccine molecule (approximately 1,000 kDa) to enter the capillary. Polysaccharide- specific antibodies were used to avoid cross-reactivity between some of the serotypes. The team evaluated the CRM197-conjugated vaccine in all 15-serotype monovalent bulk conjugates as well as in the final container. **FIGURE 32** shows the results for the vaccine mixture in the final container and indicates that each serotype is recognized by the specific antibody in the 15-valent final container. The assay was performed within 24 hours for all 15 serotypes plus CRM197 with minimal manual involvement by the analyst. Sample preparation plus loading samples and reagents onto the instrument took approximately 2 hours. ELISAs or traditional Western dot blots take 2 to 3 days to analyze 15-valent pneumococcal CRM197-conjugated vaccine and require a significant amount of hands-on labor. Manual dot blots were also performed for these samples with a relatively high failure rate. The Simple Western assay met WHO guidelines with good sensitivity and high specificity. The authors stated that the Simple Western assay provides the benefits of speed and higher throughput with 96 parallel capillaries, and its full automation reduces operator error and benchwork. They added that the assay is amenable for assay transfer to other laboratories for global vaccine production.

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DID YOU KNOW?

Peggy Sue and Sally Sue let you run high-throughput immunoassays and automate analysis for up to 96 samples at a time.

Don't need that kind of throughput? Try Jess — she'll do the same on up to 25 samples in just three hours.

REFERENCES

1. Automated capillary Western dot blot method for the identity of a 15-valent pneumococcal conjugate vaccine, M Hamm *et al.*, *Anal Biochem,* 2015; 478:33–9.

HIGH-THROUGHPUT VACCINE SEROTYPE IDENTIFICATION (CONTINUED)

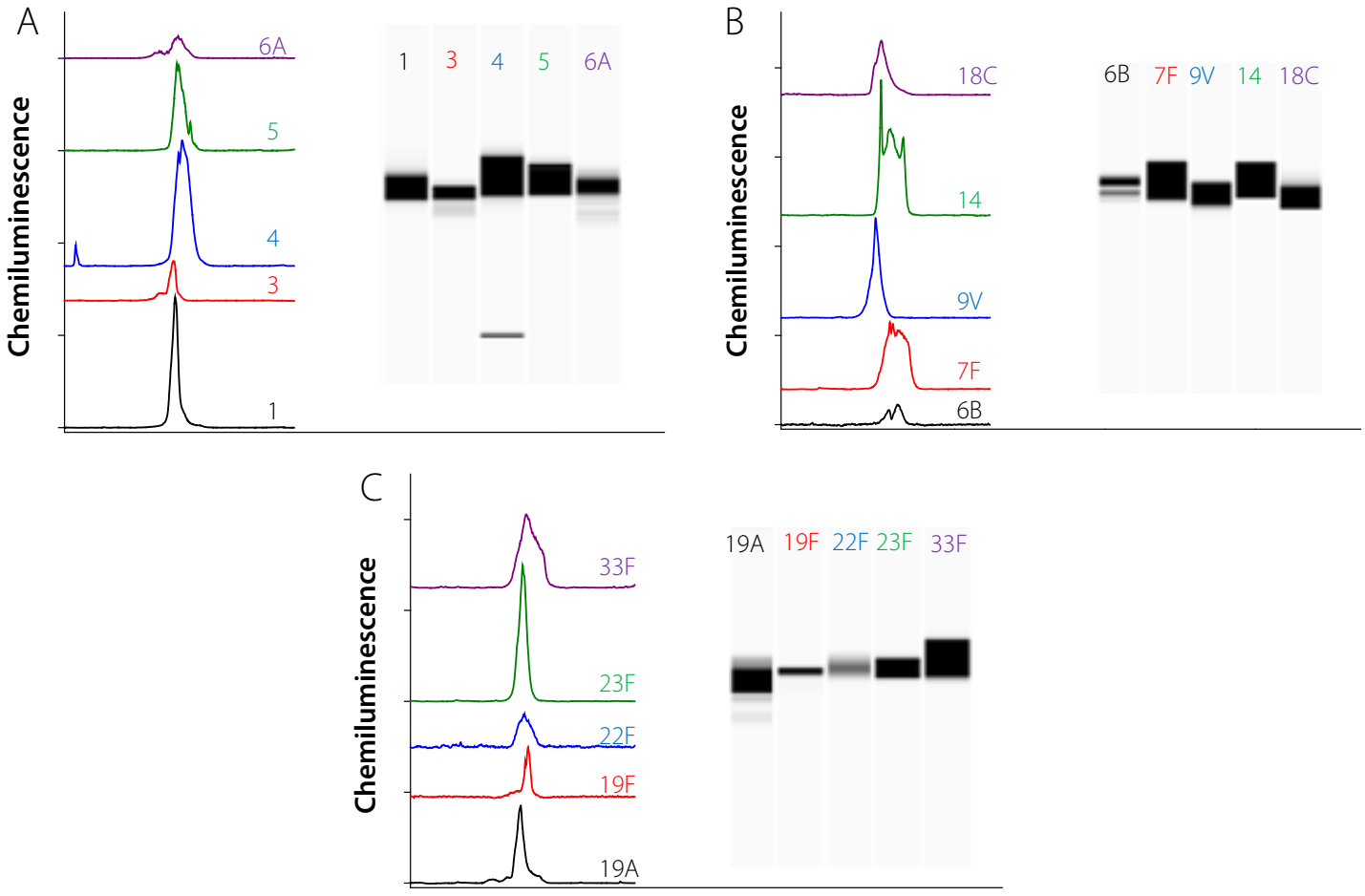

FIGURE 32: Identity test for each polysaccharide conjugated to CRM197 in the 15-valent pneumococcal conjugate vaccine final container on Peggy. (A) serotypes 1, 3, 4, 5 and 6A; (B) serotypes 6B, 7F, 9V, 14 and 18C; (C) serotypes 19A, 19F, 22F, 23F and 33F.

DID YOU KNOW?

We offer custom method development on Simple Western™ and Maurice™.

CHARACTERIZE MORE PARTICLES IN INJECTABLE PRODUCTS

The MFI™ 5000 Series system fills in the gaps that light obscuration (LO) leaves behind. You'll get the size, count, and shape of subvisible particles in the 1 µm to 300 µm range you need. The big difference? MFI's image-based detection lets you classify proteinaceous and non-protein particles like silicon oil and protein aggregates in your samples.

Supporting techniques are required to augment LO measurement of subvisible particles mandated by USP 788's limits for particles >10 µm and >25 µm. This is because LO cannot distinguish between certain particle types such as silicon oil and protein aggregates which are critical for the stability and purity assessments of injectable products. In 2011, a joint regulatory/industry panel listed Micro-Flow Imaging (MFI) as a supplementary technique to LO for particle sizing and stated that its morphologic assessment capability can be used for product characterization.[1]

Scientists at Amgen developed a biosimilar (ABP 501) to the recombinant monoclonal antibody (rmAb), adalimumab. Guidance documents dictate the need for comprehensive and in-depth physicochemical and biofunctional characterization of proposed biosimilars. Observed analytical differences must be characterized with orthogonal methods to confirm the data and to further understand differences. The team at Amgen performed a comprehensive analytical characterization of ABP 501 to determine its similarity with FDA-licensed adalimumab (adalimumab [US]) and European Union (EU)-authorized adalimumab (adalimumab [EU]), referred to as adalimumab RPs. Particles and aggregates in ABP 501 and adalimumab RP samples were analyzed by LO and MFI for the quantity and morphology of particles and aggregates. For each sample test, the particle concentration results were reported as cumulative particle counts per mL for ≥2, ≥5, ≥10 and ≥25 µm size ranges by both LO and MFI. MFI was also used to analyze ≥5 µm non-spherical particles with an aspect ratio of <0.70 (**TABLE 5**). Particle concentrations determined for all sizes were comparable for ABP 501 and adalimumab RPs.[2]

Injectable drugs may have traces of silicon oil that has leached off manufacturing equipment. The Amgen team was able to define the ratio of spherical particles to amorphous particles using MFI's image-based analytics, which gave them the ability to judge the contribution silicon oil made to the overall particle load. Morphological image analysis indicated that most of the particles present in the final product were silicon oil droplets in addition to low levels of nonspherical particles that were presumed to be proteinaceous. This was consistent between the originator molecule and the biosimilar. Had LO been the only analysis performed, the lack of this information could have led to a mischaracterization of the silicon oil as protein aggregates. Identification of two distinct particle populations within the formulation provided additional insight into the stability and safety of the product.[3]

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ANALYTICAL TESTING/ ATTRIBUTES	ABP 501 [RANGE (N)]	ADALIMUM-AB (US) [RANGE (N)]	ADALIMUM-AB (EU) [RANGE (N)]
LO/PARTICLES SIZE (PARTICLES/ML)			
≥2 µm	5,140–23,748 (10)	4,560–31,000 (7)	9,447–15,820 (7)
≥5 µm	1,000–7,630 (10)	1,057–13,600 (7)	3,577–7,587 (7)
≥10 µm	93–1,525 (10)	107–3,727 (7)	570–2,284 (7)
≥25 µm	0–14 (10)	4–97 (7)	3–60 (7)
MFI/nonspherical particles size ≥5 µm (particles/mL)	24–172 (10)	18–139 (7)	7–183 (7)

TABLE 5: LO and MFI results for the Amgen AB501 biosimilar and the adalimumab RPs. Both techniques provided similar results, however the morphological information obtained with MFI indicated that most particles greater than 5 µm in the products were silicon oil.

REFERENCES

1. Analysis and immunogenic potential of aggregates and particles, a practical approach, Part 1, A Mire-Sluis *et al*., *Bioprocess International,* 2011; 9(10):38–47.
2. Assessing analytical similarity of proposed Amgen biosimilar ABP 501 to Adalimumab, J Liu *et al*., *BioDrugs*, 2016; 30(4):321–38.
3. See what light obscuration misses with micro-flow imaging, ProteinSimple White Paper.

QUANTITATE CELL ACTIVATION BEADS IN CAR-T PRODUCTS

If you're manufacturing a CAR-T cell product for immunotherapy, Micro-Flow Imaging™ will help you meet the requirements for removing polystyrene activation beads. Personalized cell therapy with CAR-T cells involves isolation of T cells from the patient and cell activation with antibody-coated beads. MFI provides image-based sub- visible particle analysis for biopharmaceuticals with 21 CFR Part 11-compliant software.

We analyzed separate preparations of T cells and CD3/CD28 Dynabeads® for particle count and size. MFI View System Software (MVSS) automatically stores images of all particles and provides particle counts and morphological data for each imaged particle. We demonstrated reproducible T cell counting with MFI by creating a serial dilution series and analyzing each dilution. The ability to accurately quantify the number of cells is important for process development, lot release, and final product QC. We showed sensitive polystyrene activation bead counting with MFI by analyzing serially diluted bead suspensions. The ability to reproducibly quantify low numbers of beads in a CAR-T sample is critical for lot release.

After data acquisition, Image Analysis software can be used to intuitively design filters to differentiate particles based on 10 different morphological parameters (see the MFI Image Analysis User Guide). Using the MFI-measured shape parameters for subvisible particles, we developed filters to differentiate T cells and Dynabeads. Dynabeads have a well-defined equivalent circular diameter (ECD) of 4.66 ± 0.66 µm. By contrast, T cells have a larger ECD of 5 µm to 20 µm. This difference was used as a basis for building a filter in combination with circularity and intensity standard deviation to define T cell and Dynabead subpopulations (**FIGURE 33**). These parameters are not exhaustive, and filters may be tailored for any particle type. For example, layers can be added or removed, and parameter limits can be tuned for each filter. We applied this filter to the analysis of samples containing T cells and activation beads. The ability to distinguish the two populations is shown in **FIGURE 33**.

We examined if the presence of T cells impacts the ability of MFI to reproducibly count Dynabeads. To do so, we prepared a dilution series of Dynabeads with a constant number of T cells (105/mL). Then, we analyzed each dilution on MFI as summarized in **FIGURE 34**. This analysis shows that MFI can count as few as 1 to 10 beads in 1 mL of sample that is replete with cells. The ability of MFI to directly image subvisible particles and measure shape parameters enables the independent counting and characterization of T cells and activation beads in a mixed population. MFI can detect low numbers of T cells and activation beads, and the presence of T cells does not affect the counting of activation beads. Easy-to-use software filters can define sample purity for process development and final release. MFI is a valuable tool for both incoming and outgoing quality control of materials.

Explore our Bioprocessing - Final Product Characterization resources.

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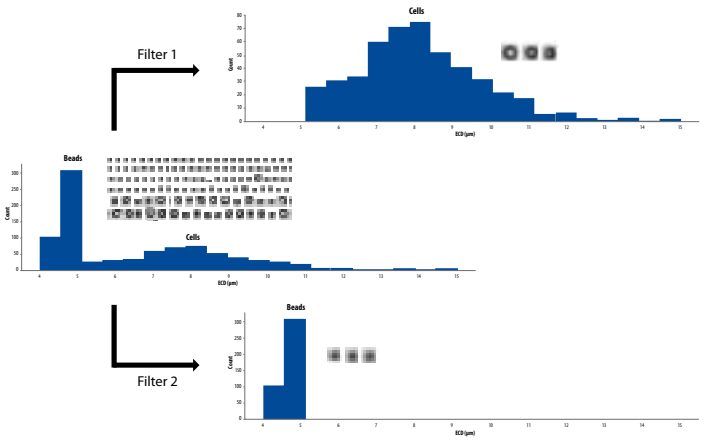

FIGURE 33: MFI can distinguish beads from cells in a mixed population.

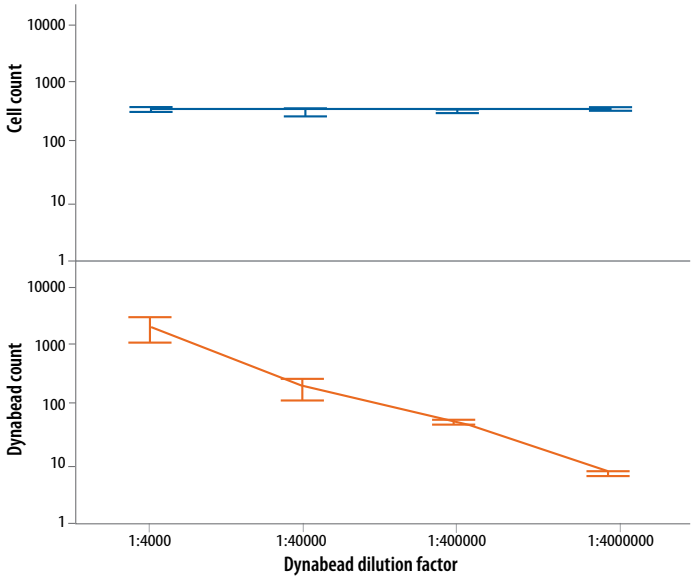

FIGURE 34: MFI can identify and count small numbers of Dynabeads in the presence of T cells. The counting of beads was unaffected by the presence of cells. A linear decrease in Dynabead count with dilution factor was observed despite the presence of T cells, and a reproducible number of Dynabeads was counted for each dilution

BETTER INFORMATION ON PROCESS RESIDUALS

Custom-developed antibodies and Simple Western™ assays detect bioprocess residuals with superior specificity. It's critical that process residuals don't affect your product purity. Simple Western size assays more than one-up other methods used to detect and identify residuals. They give you the specificity to detect co-migrating proteins, report molecular weight, and automate the entire analysis process. Bovine serum albumin (BSA) is a major component of fetal bovine serum (FBS) which is typically used in cell culture media during vaccine production. Because BSA can cause allergic reactions in humans, the World Health Organization (WHO) has set a guidance of 50 ng or less of residual BSA per vaccine dose. Standard sandwich ELISA is still the most commonly used method in the biopharmaceutical industry to analyze residual BSA, but it does not provide molecular weight (MW) information. Manual Western blotting provides MW information and the antigen specificity recognition of an immune-based assay, but it's labor intensive and qualitative in nature.

John Loughney and his colleagues at Merck Research labs developed a Simple Western size assay for quantitating residual BSA in four vaccine final drug substance batches. Figure 35 shows the Western Image view and electropherogram traces of BSA detected in the vaccine batches. With this assay, the Merck team was able to compare the four batches of vaccine for the amount of monomeric and dimeric BSA and also BSA degradation. BSA degradation was not distinguishable by ELISA. The Simple Western size assay was also used to monitor BSA clearance in process intermediate vaccine samples from clarified bulk to low salt bulk. The authors were able to test intermediate vaccine samples with less sample matrix interference compared to ELISAs. In addition, theystated that the assay allows determination of the monomeric BSA concentration, while dot blot and ELISA can't discriminate between monomeric and aggregated BSA.

The optimized Simple Western size assay has a linearity of two logs and high enough sensitivity to meet the WHO guidelines. Its limit of quantitation (LOQ), accuracy, and reproducibility are comparable to standard ELISA. However, the Simple Western method provides three benefits: (1) additional specificity by size separation, (2) elimination of matrix interference or BSA loss due to the denaturing and reducing assay conditions, and (3) full automation, resulting in increased precision and reduced hands-on time.

MEET SALLY SUE

WATCH JESS VIDEO

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REFERENCES

1. Residual bovine serum albumin (BSA) quantitation in vaccines using automated Capillary Western technology, JW Loughney *et al*., *Anal Biochem*, 2014; 461:49–56.

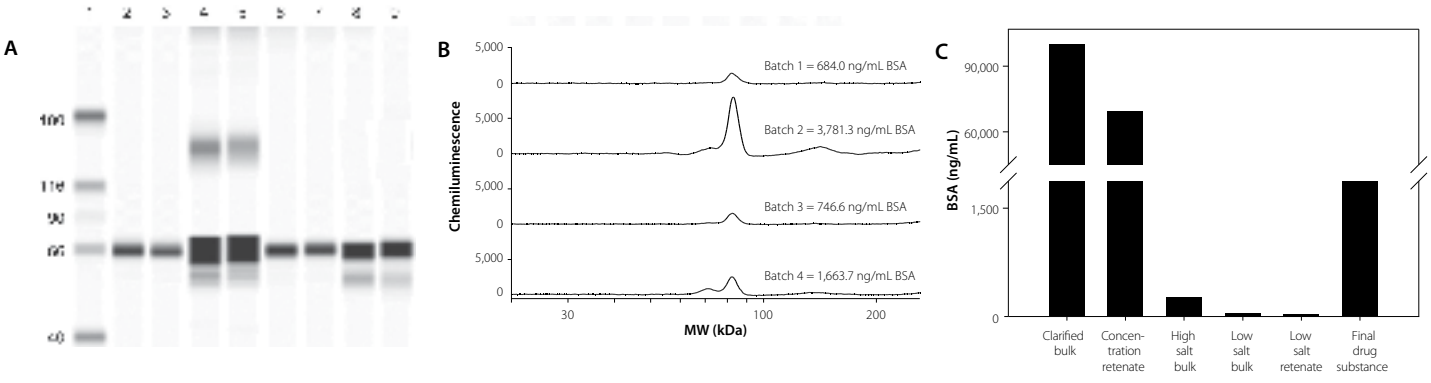


FIGURE 35: Quantitation of residual BSA in viral vaccine samples. (A) Western Image view of four vaccine final drug substance batches tested in duplicate (lanes 1, MW ladder; 2–3, Batch 1; 4–5, Batch 2; 6–7, Batch 3; 8–9, Batch 4) and (B) electropherogram traces of final drug substance batches 1–4 with dilution corrected concentrations of BSA (ng/mL). (C) Quantitative analysis of BSA concentration in process intermediate vaccine samples to demonstrate clearance of BSA.

FULLY AUTOMATED HOST CELL PROTEIN MEASUREMENTS

An important requirement during recombinant therapeutic protein or biologics production is to reduce the amount of host cell proteins (HCPs) and other contaminants like protein A or endotoxins at every step of the purification process. The FDA recommends the concentration of HCPs be less than 100 ppm in the final drug substance. This is necessary as HCPs can cause an adverse immunological reaction or other off-target effects, as well as impact drug efficacy and jeopardize the stability of the product.

There is a growing need for biopharmaceutical companies to obtain results in a more expedient manner and with less manipulation. The ability to achieve accuracy and reproducibility are also critical, as HCP assays are typically run by different scientists across different labs and facilities.

PARTNERS IN PROCESS DEVELOPMENT

To bring you the best quality bioprocess-related impurity immunoassays, we've partnered with the industry leader in impurity testing, Cygnus Technologies. Cygnus Technologies provides the highest level of validation and characterization of the antibodies in their assays, ensuring broad host cell protein coverage and reproducibility. Cygnus' orthogonal method characterization, alongside the automation with the Ella platform provides the perfect tool for process development and quality control laboratories.

CHO HCP SIMPLE PLEX™ ASSAY, 3G-1

For analyzing bioprocess samples containing Chinese Hamster Ovary Host Cell Proteins (CHO HCP), the 72x1 Simple Plex cartridge provides triplicate results for up to 72 samples. All the reagents for the assay are included in the cartridge, and the execution of the assay is completely automated using microfluidics. The cartridges are also factory calibrated which obviates the need to run standard curves. These features greatly reduce the operator time and the complexity associated with running immunoassays. Results are obtained in just 90 minutes, including assay setup time, representing a significant time and labor savings over traditional ELISA.

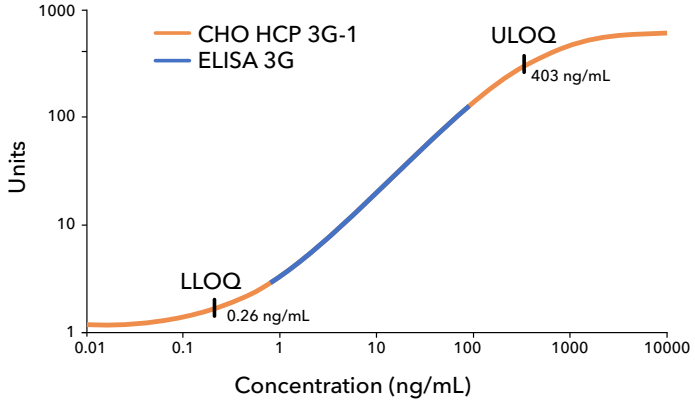
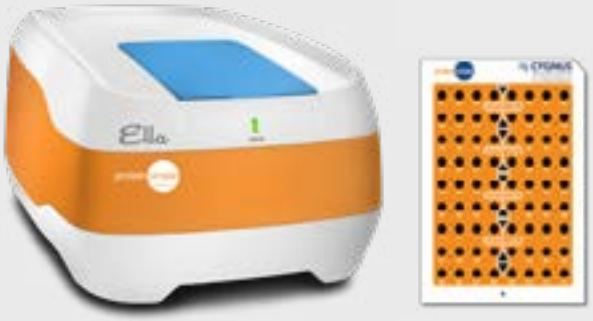


FIGURE 36: Simple Plex CHO HCP assay performance. (A) Standard curve for the Simple Plex CHO HCP assay. (B) Intra- and inter-assay variability as it was assessed during assay validation.



MEET ELLA

DID YOU KNOW?

Want molecular weight information on your HCPs? If you typically look at <20 process contaminants per sample, try Simple Western™ size assays on Sally Sue. She'll let you detect Protein A too.

Cygnus Technologies' 3rd Generation CHO-HCP ELISA Kit utilizes antibody that has been evaluated for reactivity to more than 1,000 individual HCPs present in CHO strains by state-of-the-art Antibody Affinity Extraction™ (AAE™) and mass spectrometry methods. The Simple Plex CHO-HCP assay is based on the same antibodies currently used in the Cygnus CHO HCP ELISA Kit, 3G.

AUTOMATED HIGH-QUALITY CHO HCP DATA

The assay's 3 log dynamic range helps to minimize sample dilution requirements during optimization (**FIGURE 36**). The automation and standardization of the assay and platform also help to improve intra- and inter-assay CVs. During this study intra-assay CVs were better than 9% and inter-assay CVs were better than 14.4%, showing that the assay was highly reproducible across multiple operators and days.

PARAMETER	LOW QC	HIGH QC
Intra-Mean (ng/mL)	3.12	158
Intra-assay SD	0.217	14.0
Intra-assay CV (%)	6.9	8.9
Intra-Mean (ng/mL)	3.42	162
Intra-assay SD	0.343	23.4
Intra-assay CV (%)	10.0	14.4

QUANTIFYING INDIVIDUAL IMPURITIES IN A THERAPEUTIC ANTIBODY MANUFACTURING WORKFLOW

Protein A is a 40-60 kDa surface protein originally found in the cell wall of the bacterium *Staphylococcus aureus*. Protein A affinity chromatography is used regularly in the pharmaceutical industry, as it allows for the high binding affinity and purity of monoclonal antibodies (mAbs) (1). Protein A or its fragments can leach from a purification column and remain bound to the Fc region of a therapeutic antibody. Quantifying and reducing this contaminant is critically important in instances where antibodies are manufactured for clinical uses, in order to preclude any adverse effects of residual protein A on the patient.

PROTEIN A ELISA KITS

R&D Systems has developed two ELISA kits for the reliable supply and accurate detection of Protein A residuals in the manufacturing of therapeutic monoclonal antibodies, one for natural and recombinant protein A and the other for Protein A engineered variants. These kits allow for easy transfer to automated platforms with:

- Ready-to-use components including buffers
- Single-step sample pretreatment
- Minimal assay incubations
- Consistent supply and technical support

FULLY VALIDATED FOR ACCURATE DETECTION

Multiple Protein A variants are used in the manufacturing workflow. To accurately quantify Protein A, a kit calibrated to a closely related variant must be used. Both Protein A ELISA kits have been validated and qualified based on the following parameters:

	PROTEIN A ELISA KIT	PROTEIN A ENGINEERED VARIANT ELISA KIT
Specificity	Natural Recombinant Protein A	Engineered Protein A Variants
Calibration	*E. coli* expressed recombinant Protein A	MabSelect SuRe™
Intra-Assay Precision (CV)	2.7-3.1%	2.4-3.7%
Inter-Assay Precision (CV)	6.0-8.3%	3.8-8.5%
Recovery	88-113%	84-122%
Linearity	104-112%	102-112%
Limit of Detection	16.1 pg/mL	11.4 pg/mL
Limit of Quantification	23.4 pg/mL	11.7 pg/mL

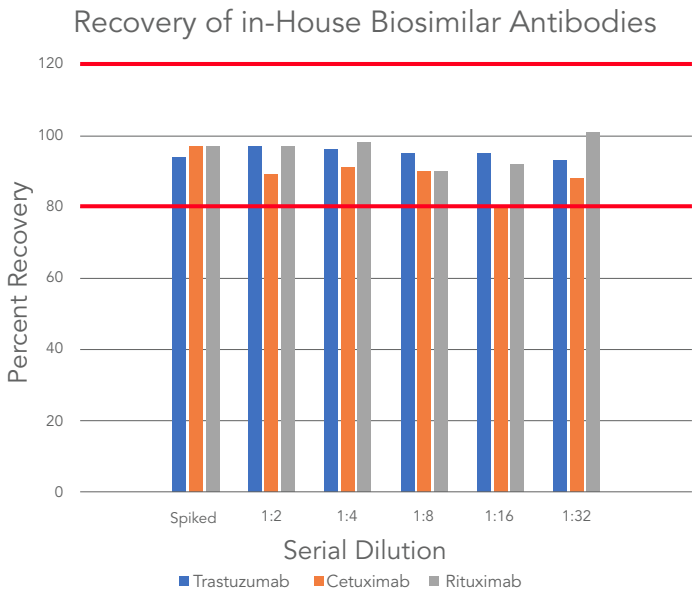


FIGURE 37: Protein A Spiked Recovery. Protein A constructs were spiked into in-house biosimilar antibodies, Tratuzumab, Cetuximab, and Rituximab and tested to measure response in the spiked sample matrix. Protein A recovery ranged from 80% to 101%, which was in the acceptable range.

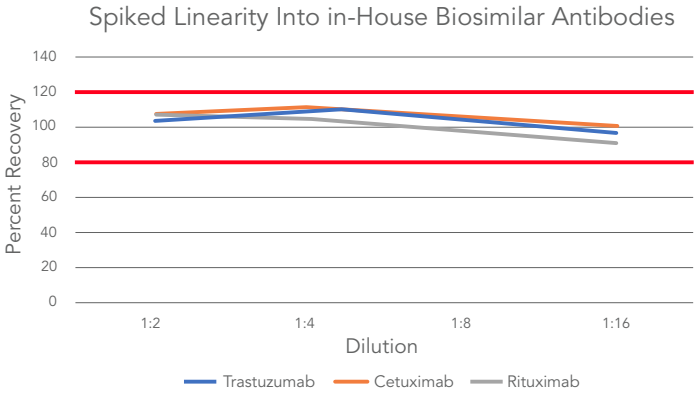


FIGURE 38: Protein A Spiked Linearity Testing. Protein A constructs are spiked at high concentrations in various in-house biosimilar antibodies and diluted with appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay. The linearity is between 97%-112%, which is in the acceptable range.

REFERENCES

1. Protein A chromatography: Challenges and progress in the purification of monoclonal antibodies. AM Ramos-de-la-Pena *et al.*, Journal of Separation Science,2019; **42**:1816-1827.

ANALYTICAL TESTING SERVICES

We offer a large collection of state-of-the-art analytical systems that can give you an excellent picture of the biochemical and biophysical state of your product. We will provide complete documentation for all analysis we do for your product. If you'd like to outsource this testing, we'd love to help!

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- Interfaces with UPLC and fluorescence detector to permit glycan mapping
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- Differential chromatographic analysis increases call confidence.
- De-blocking capability with our Recombinant Human PGPEP-1.

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- Data can be queried against a database of known retention times to identify N- glycans liberated from protein and antibody surface.
- Excellent for lot-to-lot consistency testing, biosimilar/ originator comparisons, or as a companion to mass spectrometry.

DYNAMIC LIGHT SCATTERING (DLS)

- Powerful companion instrument to MFI and SEC, capable of detecting very low levels of high molecular mass and soluble protein aggregates in solution.
- Lower resolution than SEC but eliminates the risk of false negatives due to SEC "filtration".

TANDEM DLS AND SEC FOR STATIC LIGHT SCATTERING APPLICATIONS

- By combining size exclusion chromatography with dynamic light scattering, solution state molecular mass can be calculated for proteins and antibodies.
- Right angle light scattering on chromatographically resolved proteins and antibodies. Provides mathematical calculation of shape-independent solution state mass calculation.

PROTEINSIMPLE MICRO-FLOW IMAGING™ FOR PARTICLE ANALYSIS

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