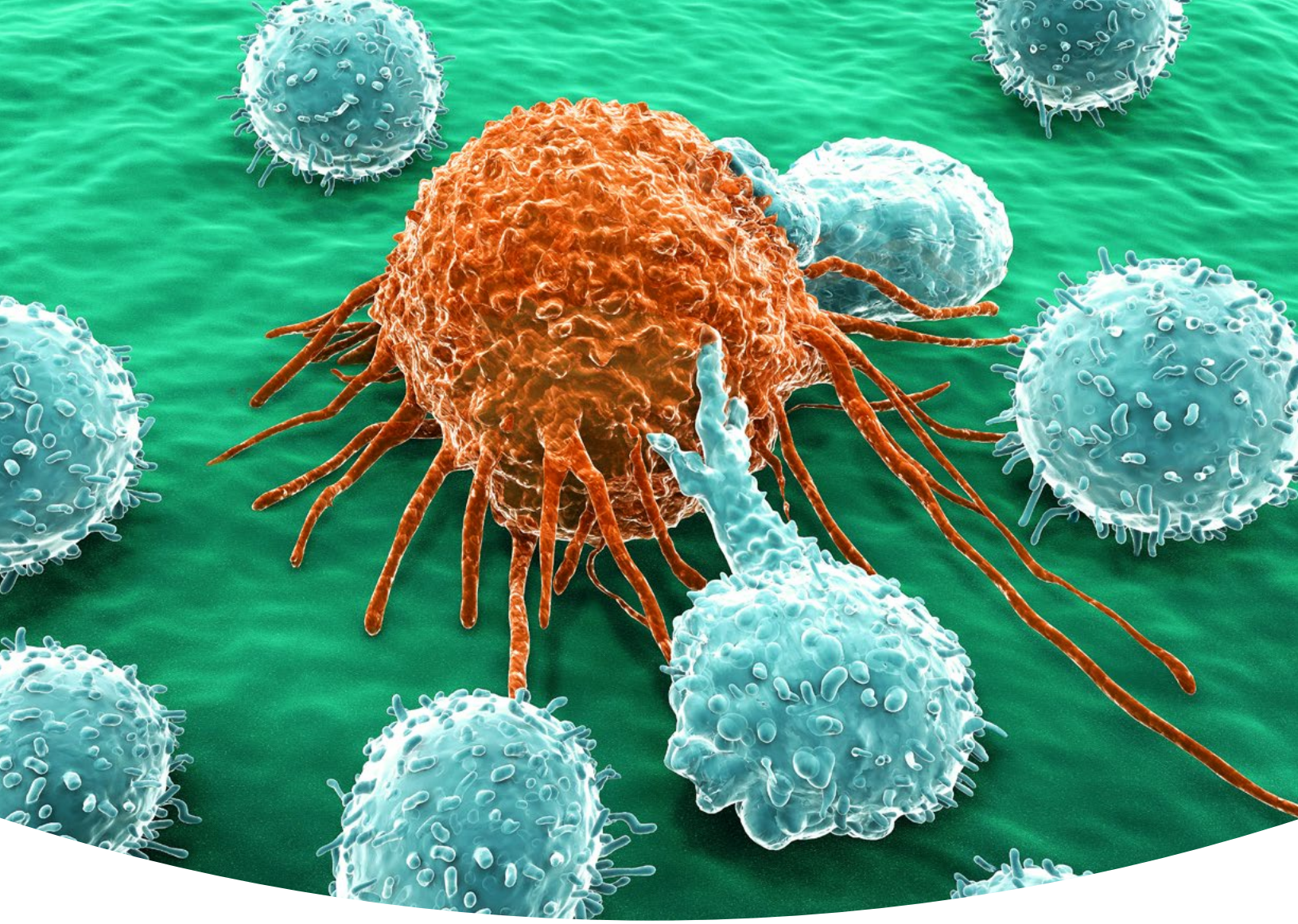

Investigating Immuno-Oncology: Advances in Protein Analysis Tools

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Contents

CHAPTER 1
Introduction

The tumor microenvironment is a site of complex cellular interactions between tumor cells and other non-cancerous cells, including fibroblasts, lymphocytes, blood vessels and immune cells. Whereas the role of transformed cells in tumorigenesis and metastasis has long been under investigation, researchers are now focusing on dynamic interactions between tumor and immune cells and developing approaches to harness the power of immune cells as a therapeutic strategy. This immuno-oncology approach offers new hope for targeted treatment. In this eBook, we examine technological advances in protein analysis that are at the forefront of immuno-oncology research and therapeutic development.

Immuno-oncology has come a long way from its origins with the pioneering work of Coley[1] and Ehrlich[2]. Since the launch of the first immuno-oncology drug, rituximab in 1997, a number of strategies have been developed and tested. These include other monoclonal antibodies that target the immune system, as well as cytokine treatments, cell-based therapies and chimeric antigen receptor (CAR) T-cell therapy. Many other agents are under clinical investigation including checkpoint inhibitors, oncolytic viruses, antibody-drug conjugates, small molecules, adjuvants, vaccines, bi-specific antibodies and dual specific antibodies. For a history of immuno-oncology drug development and current treatment modalities see the review by Axel Hoos[3].

As the field of immuno-oncology moves rapidly from bench to bedside there is a need for improved techniques to study and analyze complex cellular interactions and dynamic networks of signaling pathways in the tumor microenvironment. Limited clinical samples, heterogeneity in cellular behavior, complex collection paradigms and the personalization of treatment strategies require approaches that address R&D questions faster and more efficiently.

At ProteinSimple we're changing the way researchers analyze proteins. Our innovative product portfolio helps researchers reveal new insight into proteins, advancing their understanding of protein function. We enable cutting-edge research to uncover the role of proteins in disease and provide novel approaches to develop and analyze protein-based therapeutics. We empower you to make your next discovery by eliminating common protein analysis workflow challenges. From basic research to quality testing and formulation, you can think of us as your immuno-oncology research partner.

Click here to learn more about ProteinSimple.

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2. Über den jetzigen stand der karzinomforschung. P Ehrlich, *Ned Tijdschr Geneeskd*, 1909; 5:273–290.
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Click here to meet Sally Sue

Click here to meet Peggy Sue

Click here to meet Wes

Click here to meet Ella

CHAPTER 2

The search for reproducibility and reliability: assays you can trust

Are your assays lacking the reproducibility and reliability that you need to be confident in your research? Don't waste resources, time and money with assays and techniques that aren't reliable. Let the ProteinSimple approach put you on the road to success.

What challenges do you face?

1. My current method isn't reproducible

The foundation of any scientific result is that it must be reproducible. Traditional Westerns are as much an art form as they are a technical tool. They are rarely reproducible, can take years to master and after you've worked out the conditions they work beautifully until they don't — leaving you wondering what went wrong. Getting good immunoassay reproducibility is tricky with all the multiple, manual steps. Reducing the number of assay steps requiring reagent additions and manual interventions in your protein assays saves time and can decrease the risk of error which translates to more reproducible results you can rely on.

Serum biomarkers are commonly used as indicators for the diagnosis and treatment of cancer. Traditional Western blots and ELISAs are often used for biomarker verification and validation, but require a lot of hands-on time. Simple Western™ and Simple Plex™ assays give you fast, sensitive, precise and reproducible information about your biomarkers of interest, with minimal manual interventions.

Simple Western assays are automated, capillary-based immunoassays that solve many of the challenges that come with traditional Westerns. There are four Simple Western systems to choose from, all produce very reproducible data with intra-assay coefficient of variations (CVs) <15%. Wes™ gives you relative quantitation on up to 25 data points in just three hours with only 30 minutes of hands-on time for setup. If you need higher throughput, Sally Sue™, Peggy Sue™ and NanoPro 1000 give you 96 data points overnight and only need about an hour of hands-on time.

Simple Plex assays, run on the Ella immunoassay platform, are a microfluidic alternative to ELISA that lets you validate serum biomarkers faster, with inter-assay reproducibility in the single-digit CVs and sensitivity in the low pg/mL. Setup takes 10–15 minutes and results are ready in just one hour, letting you screen serum samples 3–6X faster than a traditional ELISA.

To demonstrate how Simple Western and Simple Plex assays data give similar trends and work together to give you reproducible results, we ran the same samples and compared the average signal area and CV to see how well the data correlated. Six spiked serum samples were analyzed using the Simple Western assay on Wes to generate triplicate data for four targets of interest. Simple Plex assays on Ella were run using two 16x4 cartridges as different dilutions were needed to get all four targets in

	IL-6 (1:50)		BDNF (1:50)		IL-1α (1:50)		PCSK9* (1:50)	
Sample	**Average Signal**	**% CV**	**Average Signal**	**% CV**	**Average Signal**	**% CV**	**Average Signal**	**% CV**
1	145810	11.3	97837	13.4	ND	---	19170	12.9
2	191301	12.9	133003	9.6	ND	---	17340	9.1
3	ND	---	107789	1.3	8574	10.9	17334	10.2
4	35777	13.3	71049	12.8	4770	9.2	18513	4.6
5	8305	10.2	118950	10.8	14278	2.0	16780	12.1
6	77614	3.2	88094	5.4	12052	7.5	18692	8.6

TABLE 1. Relative quantitation of spiked IL-6, BDNF, IL-1α, and endogenous PCSK9 in six normal human serum samples using Wes. Samples were all diluted 1:50 in 0.1X Sample Buffer and 5X Fluorescent Standard and heat denatured at 95 °C for 5 minutes. Triplicate data was generated in one day and was extremely reproducible with CVs all under 13.4%. *Endogenous protein detected. ND = not detected.

	IL-6 (1:1000)		BDNF (1:100,000)		IL-1α (1:10)		PCSK9* (1:10)	
Sample	**Average Concentration (pg/mL)**	**% CV**	**Average Concentration (pg/mL)**	**% CV**	**Average Concentration (pg/mL)**	**% CV**	**Average Concentration (pg/mL)**	**% CV**
1	21,693	5.2	136,074,206	2.0	83	1.6	140,052	2.0
2	39,905	2.0	227,185,347	2.5	26	1.5	143,325	1.0
3	809	9.5	156,588,495	2.1	2,562	2.3	137,668	2.1
4	8,770	9.0	123,014,794	2.8	970	6.6	162,587	2.8
5	1,377	1.2	175,274,056	1.3	5,205	0.8	137,478	1.3
6	26,153	6.2	125,021,865	1.8	2,463	0.5	157,494	0.8

TABLE 2. Absolute quantitation of spiked IL-6, BDNF, IL-1α, and endogenous PCSK9 in six normal human serum samples using a Simple Plex assay on Ella. Samples were diluted in SD13 Sample Buffer at 1:10, 1:1000, or 1:100,000 before loading on the 16x4 Simple Plex cartridge. Triplicate data with single-digit CVs were generated using two cartridges in in just two hours. *Endogenous protein detected.

the linear, quantitative range. With Wes, one sample had levels of IL-6 below the limit of quantitation (LOQ) and two samples had levels of IL-1α below the LOQ. For the remaining samples, reproducibility between technical replicates was very good with all CVs ≤13.4% (**Table 1**). With Ella, % CVs were all single-digit with an average across all six samples and four targets of less than 2.9% (**Table 2**).

Vinita Gupta and colleagues from Genentech examined the analytical performance of cytokine Simple Plex assays on Ella.[1] They calculated an inter-assay CV across six runs of <15% and concluded that "…[t]he qualified assays will be useful in assessing mechanism of action cancer immunotherapies."

In an analysis of biomarkers in 21 patients with hematological malignancies undergoing allogeneic stem cell transplantation, Prathima Anandi and team members from the National Institutes of Health compared Simple Plex assays run on Ella with traditional ELISA.[2] The resulting inter-assay CV was lower with Simple Plex assays compared to ELISAs.

Jin-Qiu Chen and colleagues from both the National Cancer Institute and National Institutes of Health evaluated and reviewed the use of Simple Western technology to create molecular profiles of biomarkers for

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Application Note: Accelerated Serum Biomarker Verification and Validation with Wes and Ella. Click here to read the Application Note

WANT TO LEARN MORE ABOUT ELLA?
Click here to watch a video to see how Ella can process up to 72 samples in 75 minutes. The short video shows the step by step process, from sample loading to analyzed data.

WANT TO LEARN HOW WES WORKS?
Click here to watch a video to see how Wes can process up to 25 samples in 3 hours. The short video shows the step by step process, from sample loading to signal detection.

clinical decisions.[3] They concluded that the system "…[o]ffers easy operation, precise and accurate measurement of proteins and their post-translational modifications and a fast turn-around time" and that "…[g]ood intra-assay, inter-run and inter-person data reproducibility have been observed with the system, which enables assay standardization across multiple testing sites."

2. I need to transfer my immunoassay and get the same result

As your experiments move out of basic research and into a clinical setting you may want to transfer you assay to other labs, clinical sites or a contract research organization. Because Simple Plex immunoassays are precisely controlled in the cartridge, there's no chance for pipetting errors. You'll get the same reproducible answers across multiple users and multiple sites.

To assess the reproducibility of Simple Plex assays, 4 different assays were processed for CCL2, IL-6, TNF α and VEGF-A, using 8 unique serum samples and 2 controls. This multi-site, multi-user reproducibility test involved 3 sites, 11 users and 9 Ella instruments for a total of 704 answers. **Figure 1** shows the normalized concentration of all results in this multi-site test with consistent answers between different users, sites and Ella instruments.

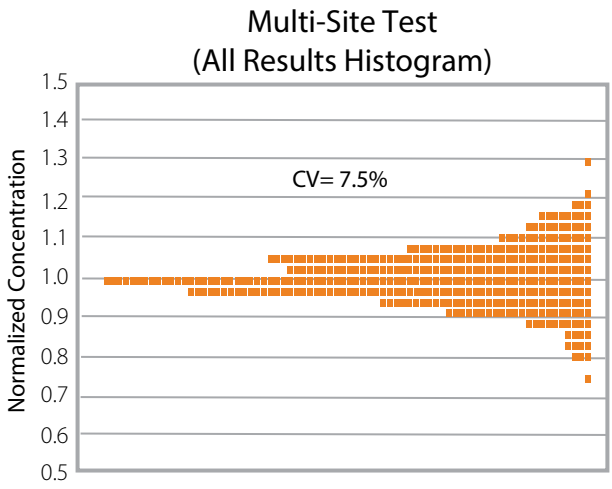

FIGURE 1. Multi-site, multi-user reproducibility test – across 3 sites, 11 users and 9 Ella instruments. Four different assays were processed for CCL2, IL-6, TNF α and VEGF-A, using 8 unique serum samples and 2 controls.

FROM YOUR PEERS

"Ella allows us to rapidly quantify biomarkers from hundreds of cryopreserved samples and correlate them to specific outcomes. Now we're ready to validate that what we identified in frozen plasma samples correlates to fresh blood samples taken at the point-of-care. Ella's simplicity and reproducibility will allow us to reliably test samples across different sites, enhancing triage especially in remote locations of developing countries where access to trained healthcare professionals is unlikely."

— Aleks Leligdowicz, M.D./Ph.D., Department of Medicine, University of Toronto

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3. My assays are error-prone

All of the multiple, manual steps that come with traditional protein analysis methods make getting good assay reproducibility out of reach. They can be tedious, labor intensive and error prone. Methods that reduce the number of steps and the associated hands-on work can improve overall assay reproducibility.

With Ella, everything happens in the Simple Plex cartridge so each immunoassay is run the same way every time. There are no manual steps, washes or reagent additions. The volume on every assay is precisely controlled, so every sample is handled the same way — removing the variability that typically comes with pipetting error volume discrepancies.

Simple Western systems reinvent how Western blots are done, automate all assay steps from protein separation, immunoprobing, detection and analysis of data. They also deliver quantitative, reproducible data in hours instead of days. There's no cutting of individual strips, repeated washing steps and incubations. With Simple Western assays, you just pipette your sample and reagents into the wells of an assay plate, set up your run and press start.

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Simple Western

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Simple Plex

Click here to learn more about Ella

Looking for flexibility with your plex? Click here to see assays

Looking for pricing? Request a quote

FROM YOUR PEERS

"The no-gel, no-transfer, no-membrane features are beneficial for the reproducibility of the assay. I really like this system over traditional Western."

— Kazuya Machida, M.D., Ph.D., Associate Professor, Department of Genetics and Genome Sciences, UConn Health

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Click here to meet NanoPro 1000

Click here to meet Wes

Click here to meet Ella

Click here to meet Milo

Chapter 3

Getting the most out of your immuno-oncology samples

Do you suffer from a fear of missing out (FOMO)? Missing the limits of detection of your protein(s) of interest, missing out on the best use of your samples, missing out on analyzing multiple proteins of interest when you don't have enough samples? Don't let FOMO hold you back when it comes to protein analysis of your samples. Take back control! Learn how the latest technological innovations can simplify your research and maximize the use of your sample.

What challenges do you face?

1. I'm working with limited samples

A lot of hard work went into the planning and execution of your experimental paradigm, which makes your research samples precious. Often the small amounts of clinical sample collected or even your protocol may limit repeat analysis. No matter what your restrictions are from biomarker analysis to tumor profiling, you always want to get the maximum use out of your samples.

Shu-Bai Liu and colleagues from Brigham and Women's Hospital and Harvard Medical School used the Simple Western Size Assay to monitor cell signaling and biomarkers in tumor cell lines and clinical samples.[1] Using the assay, they were able to detect and compare the expression pattern of two tumor suppressors, PTEN and β-catenin, in very limited clinical tissue samples. They identified changes in expression levels and patterns compared to a cell lysate control and concluded that "…[t]he Simple Western size assay protocol has the capability to measure protein expression with higher sensitivity and with minimal assay optimization compared to traditional Westerns."

LEARN MORE

Click here to watch an on-demand webinar and learn how Dr. Mark Aspinall-O'Dea used ProteinSimple's Simple Western Charge Assay, run on the NanoPro 1000 capillary-immunoassay instrument, to develop methodologies appropriate for limited clinical sample sizes

The complexity of the tumor microenvironment presents unique challenges to immuno-oncology researchers. To analyze subpopulations of cells within this microenvironment you may need to first isolate specific cell types to create samples that are highly enriched for your cells of interest. Isolation of specific cell subpopulations can be done through flow sorting or laser-capture microdissection (LCM), both of which result in samples that may be too limited to analyze with traditional proteomic analysis tools.

Simple Western assays only need 3–10 µL of sample to measure protein abundance and/or post-translational modifications, and they do it in a capillary. Dr. David Rosenberg at the Center for Molecular Medicine at the University of Connecticut Health Center used LCM to isolate aberrant crypt foci (ACF) with KRAS and BRAF mutations associated with colorectal cancer.[2] Before implementing Simple Western assays in their research, their proteomic analysis was limited to routine histological and immunohistological analysis due to small sample size. ACF biopsies were subjected to LCM, separated using the Simple Western Charge Assay and probed for total ERK1/2, pERK1, ppERK1, pERK2, and ppERK2. ERK1 and ERK2 phosphorylation of normal ACF samples were compared to WT BRAF/KRAS ACF, KRAS, and BRAF tissue. Increased levels of pERK1 and ppERK1 were observed in WT BRAF/KRAS and KRAS tissue only, whereas pERK1, ppERK1, pERK2, and ppERK2 levels were increased in all tissue samples (**Figure 2**). Simple Western assays gave Dr. Rosenberg a method that

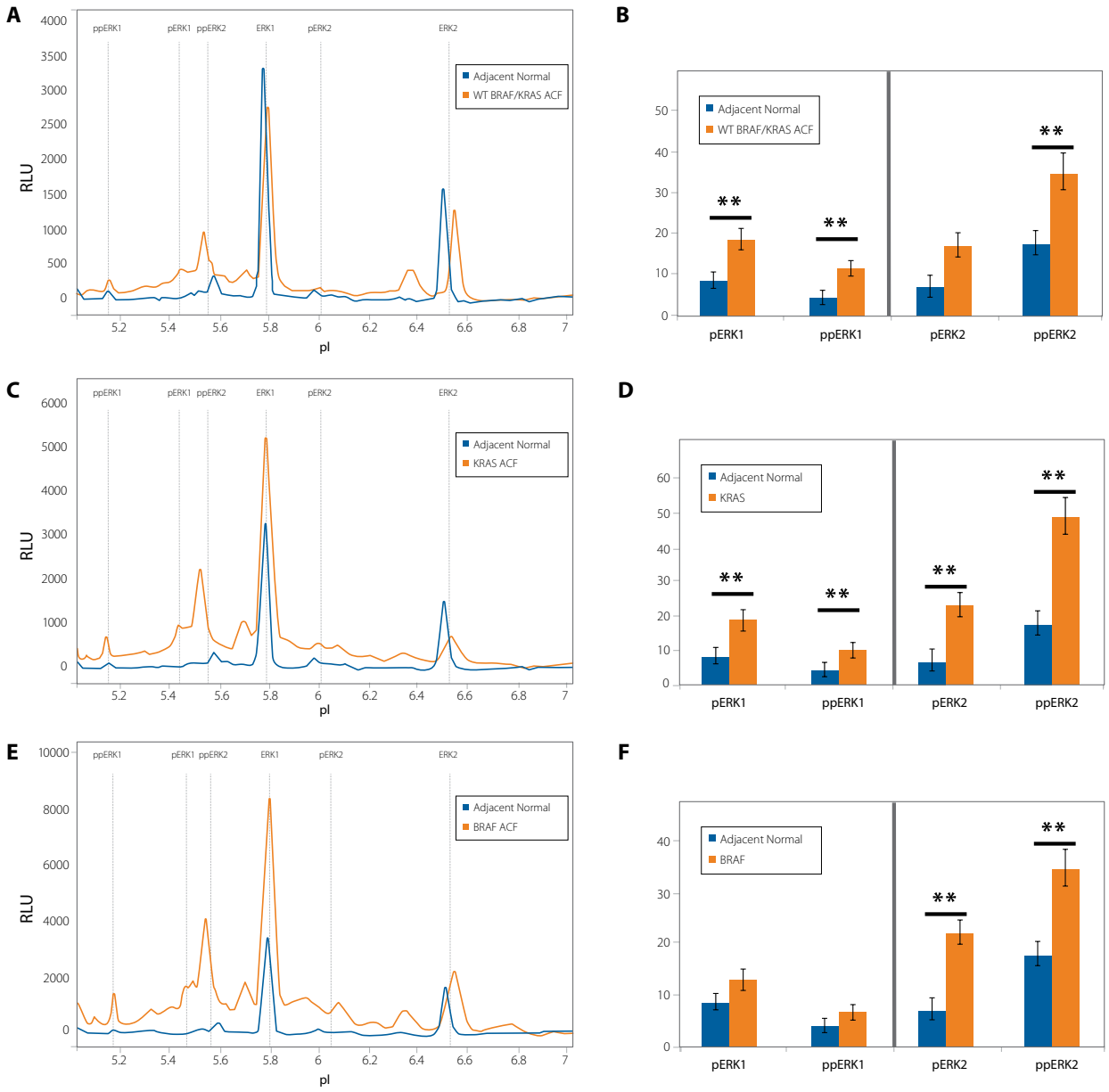

FIGURE 2. ERK1/2 detected in aberrant crypt foci (ACF). WT KRAS/BRAS (A and B), KRAS mutant (C and D) and BRAF (E and F) LCM samples (orange) were compared to adjacent normal tissue (blue) using Simple Western Charge. Phospho-ERK1 levels were only elevated in WT BRAF/KRAS and KRAS mutants while phospho-ERK2 levels were increased in all ACF samples.

increased the informative potential of a single biopsy specimen that can ultimately enable his team to finally understand disease progression in their ACF samples at a molecular level.

Highly enriched or sparingly available samples can be challenging to measure with flow cytometry because of

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Breaking Laser Capture Microdissection Sample Size Road Blocks with Simple Western

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FROM YOUR PEERS

"We collect samples from laser microdissection and were using our entire sample on just one regular Western blot. With Wes, we can do multiple assays with one sample collection."

— Mary Howell, Laboratory Coordinator, Department of Internal Medicine, East Tennessee State University

Click here to read more

low epitope availability, poor flow antibodies or challenges in assay design and implementation. The Milo™ Single-Cell Western platform enables the analysis of protein expression heterogeneity in your enriched flow sorted or sparingly available samples. As few as 10,000 cells can be loaded onto Milo's scWest chips to capture single cells for further protein expression analysis. Scientists at the University of California, Irvine used Milo downstream of their flow sorting experiments to analyze heterogeneity in human breast tissue. They used flow cytometry to enrich for Epcam+ cells from heterogeneous human breast tissue and then the sorted cell suspension was collected and analyzed on Milo to analyze cytokeratin (KRT8) expression heterogeneity. A tri-modal distribution of KRT8 expression was observed

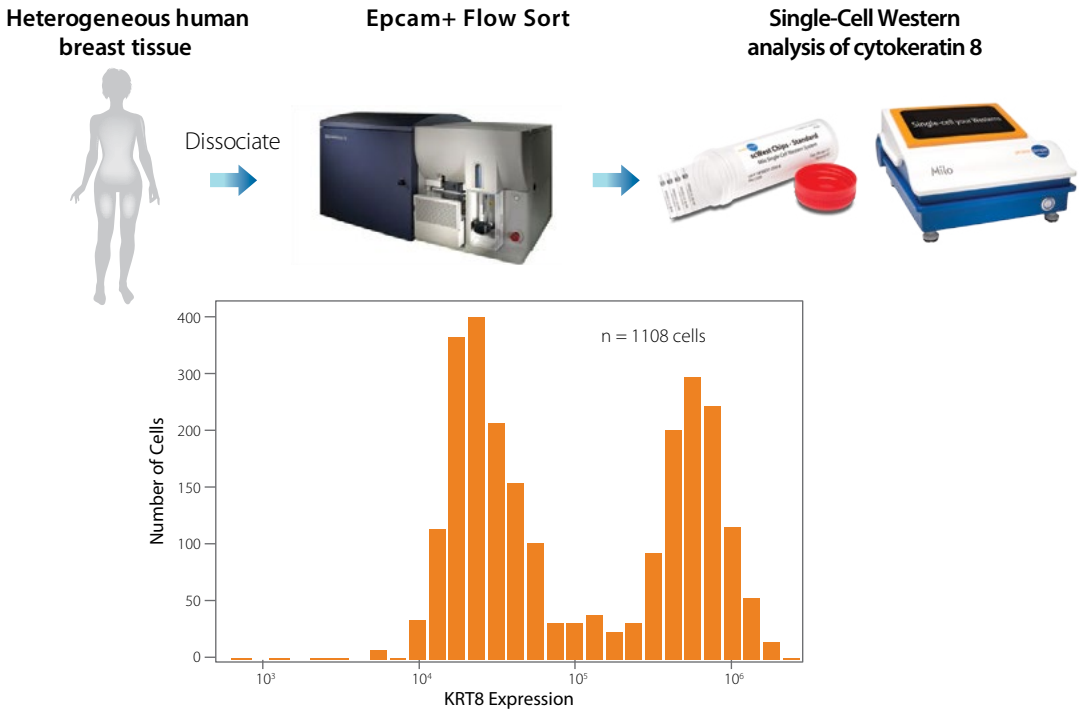


FIGURE 3. Analysis of Epcam+ breast tissue on Milo. Heterogeneous human breast tissue was dissociated, flow sorted to enrich for Epcam+ cells and then analyzed in Milo for KRT8 expression. Heterogeneity in KRT8 expression was observed — 50% of Epcam+ cells were negative for KRT8, 25% of Epcam+ cells had low KRT8 expression and 25% of Epcam+ cells had high KRT8 expression.

— approximately 50% of Epcam+ cells were negative for KRT8 while 25% of Epcam+ cells had low KRT8 expression and 25% of Epcam+ cells had high KRT8 expression (**Figure 3**). This experiment could also have been done by simultaneously probing the scWest chip for both Epcam and KRT8 to measure KRT8 expression in Epcam+ cells.

Biopsies from mice can yield excruciatingly few cell numbers which prohibit analysis with traditional protein analysis techniques such as flow cytometry or conventional Western blots. Single-Cell Westerns on Milo were used by scientists at University of North Carolina, Chapel Hill to analyze dissociated tissue biopsied from mouse small intestinal epithelium and flow sorted to enrich for Sox9-EGFP expressing cells. Cells were loaded onto scWest chips and Milo was used to measure both Sox9 and GFP expression along with a β-tubulin control in this sparingly available sample (**Figure 4A**). This analysis revealed that Sox9 expression varied more than 30-fold across the cells analyzed (**Figure 4B**).

Milo can also be used to analyze a protein panel in rare cell types such as circulating tumor cells (CTCs). Elly Sinkala and colleagues from University of California, Berkeley, used Single-Cell Westerns to detect eight protein targets in circulating tumor cells, enabling a detailed analysis of cellular profiles and the characterization of

WANT TO LEARN HOW MILO WORKS?
He measures protein expression in thousands of single cells in a single run so you can profile heterogeneity in your samples. Just load your cell suspension and the scWest chip captures ~1,000 single-cells. Milo then does a fast, 1-minute SDS-PAGE separation on each single-cell lysate on-chip. Next just probe with your favorite conventional Western antibodies to measure up to 4 proteins per cell simultaneously. Click here to watch a short video to see how Milo can automate 1,000 single cell separations in just 4 hours.

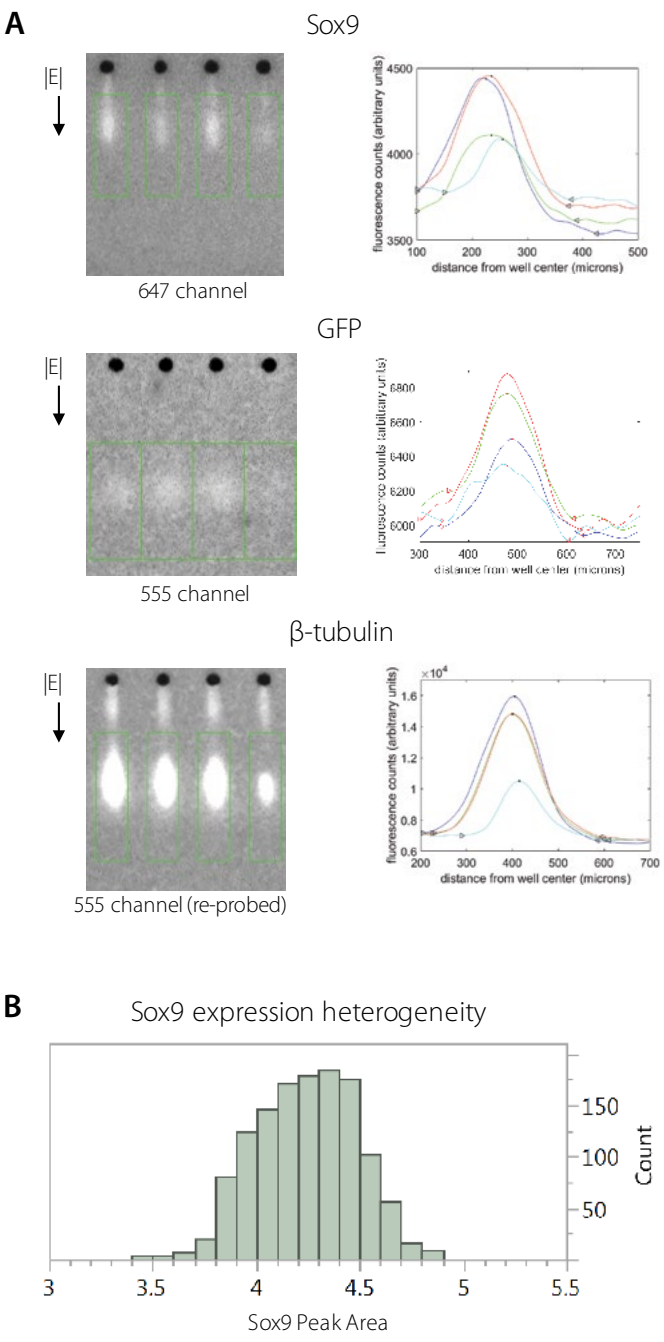


FIGURE 4. Analysis of Sox9, GFP and β-tubulin in Sox9-EGFP-sorted cells from mouse small intestinal epithelium using Milo(A). Sox9 expression varied more than 30-fold across the cells analyzed (B)

distinct biophysical phenotypes.[3] The precision and detection sensitivity of the assay eliminated the need for post-isolation cell culture and "… [o]ffers a new approach to examining CTCs, with relevance spanning from understanding CTC biology to monitoring an individual's response to therapy."

2. I need to detect endogenous protein levels

From diagnosis to prognosis, the detection and accurate analysis of biomarkers is critical to immunotherapy research and treatment paradigms. However, a major limiting factor is the ability to identify and validate biomarkers of the immune response at endogenous levels.

Immune checkpoints are a promising immunotherapy target to control cancer. Immune checkpoint inhibitors targeting molecules, like programmed death-ligand 1 (PD-L1), are being examined due to their durable effects and their ability to target a broad range of cancers with manageable toxicity compared to traditional chemotherapy. Current diagnostic tests for PD-L1 use immunohistochemistry to score the tumor microenvironment, but results can be variable. Traditional, high-quality ELISAs don't always have the sensitivity to detect endogenous levels of PD-L1 in primary patient samples (**Figure 5**). Ella's Simple Plex PD-L1 assay gets rid of these downsides, letting you detect single-digit picogram levels of endogenous PD-L1 with single-digit CVs.

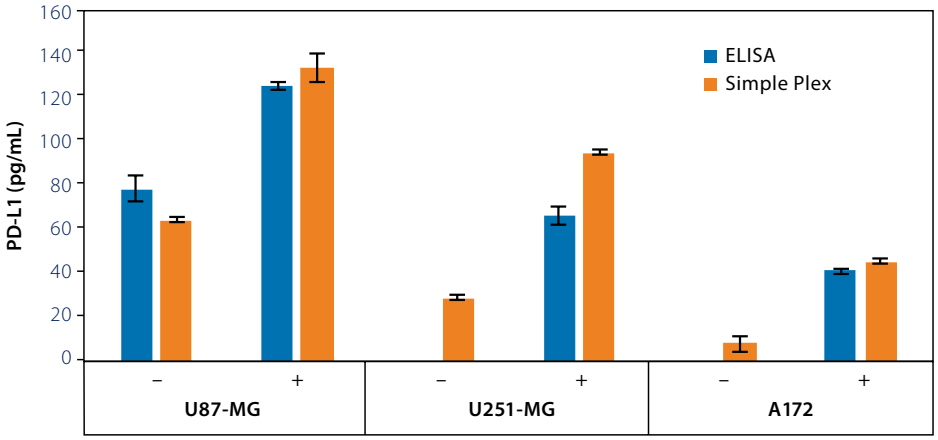


FIGURE 5. Traditional sandwich ELISA and Simple Plex assays both detect similar fold increases in PD-L1 expression after PMA treatment. The Simple Plex assay detected PD-L1 in all samples, but the traditional ELISA didn't detect PD-L1 expression in control samples below its LLOQ.

Scientists from Genentech examined the ability of the Simple Plex platform for detecting low endogenous level analytes such as IL-6, IL-8 and TNF α and concluded that the platform "…[p]resents an important tool for understanding mechanism of action immune-checkpoint inhibitors", with high sensitivity detection of low endogenous level analytes.[4] They proposed that Simple Plex assays are useful for assessing the mechanism of action of immunotherapies, and for the focused clinical validation of biomarker candidates.

LEARN MORE
Application Note: High Fidelity Detection of Endogenous PD-L1 at Low Picogram Levels with Simple Plex Assays.
Click here to read the Application Note

3. I need to detect multiple proteins in my samples

The complexity of the immune response often means you need to accurately screen and validate multiple targets in immuno-oncology research. Multiplexing can give you the flexibility to increase the number of data points per sample. Our Simple Western, Single-Cell Western and Simple Plex platforms can all multiplex so you can get the most data from your samples.

Our Simple Western solution lets you get multiplexed assays up and running quickly and efficiently. See our how-to guide to learn more along with tips to implement the workflow in your lab.

Jing Cao and colleagues at the University of Minnesota used Simple Plex assays to simultaneously detect IL-1β, TNF-α, IL-6, and IL-10 in serum samples.[5] They were able to detect these key immune regulators in clinical samples within their known analytical measurement ranges. They concluded that Simple Plex represents a promising translational research platform for the simultaneous measurement of multiple protein biomarkers in clinical samples.

LEARN MORE

Click here to watch an on-demand webinar with Greg Marusov, Senior Scientist, to learn how Luminex and Simple Plex can be used to screen and validate multiple targets accurately for immuno-oncology.

How to multiplex with Single-Cell Westerns

Single-Cell Westerns on Milo can also multiplex to measure approximately four proteins per single cell via spectral multiplexing and size-based multiplexing. To spectrally multiplex, first probe your targets of interest with primary antibodies raised in different host species — mouse and rabbit for example. Next, probe with host-specific secondary antibodies tagged with different fluorophores, for example donkey anti-mouse Cy3 and donkey anti-rabbit Cy5. For size-based multiplexing, if there's more than a 30% molecular weight difference between your proteins of interest, you can probe them with primary antibodies raised in the same host species and image them in the same spectral channel. These targets can be resolved in the SDS-PAGE separation on the scWest chip and differentiated based on molecular weight. scWest chips can also be stripped and re-probed up to 9 times for additional multiplexing. **Figure 6** shows Single-Cell Western data for four proteins detected in a single cell. Three different spectral channels were used to detect three species, while β-tubulin and L858R were resolved based on their different molecular weights.

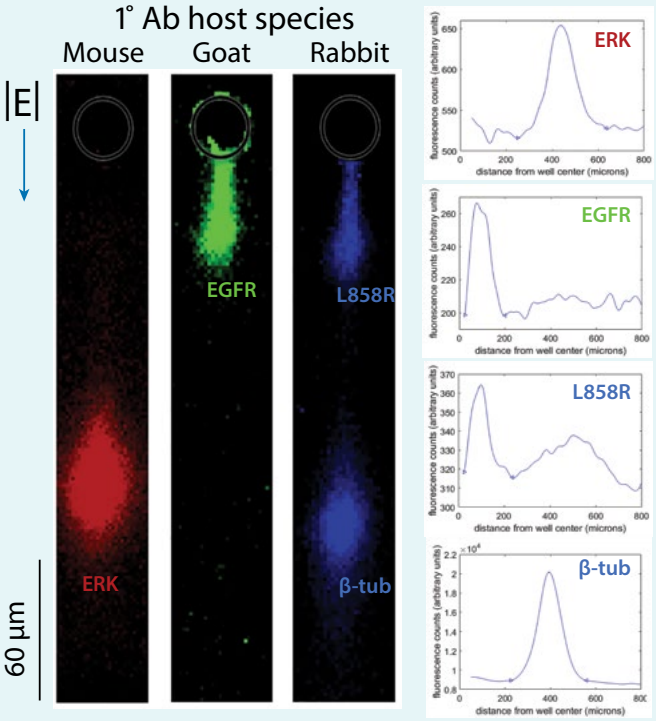


FIGURE 6. Multiplexing with Single-Cell Western. Data for four proteins detected in a single cell. Three different spectral channels were used to detect three species, anti-mouse to detect ERK, anti-goat to detect EGFP, while β-tubulin and L858R were detected with anti-rabbit and resolved based on their different molecular weights.

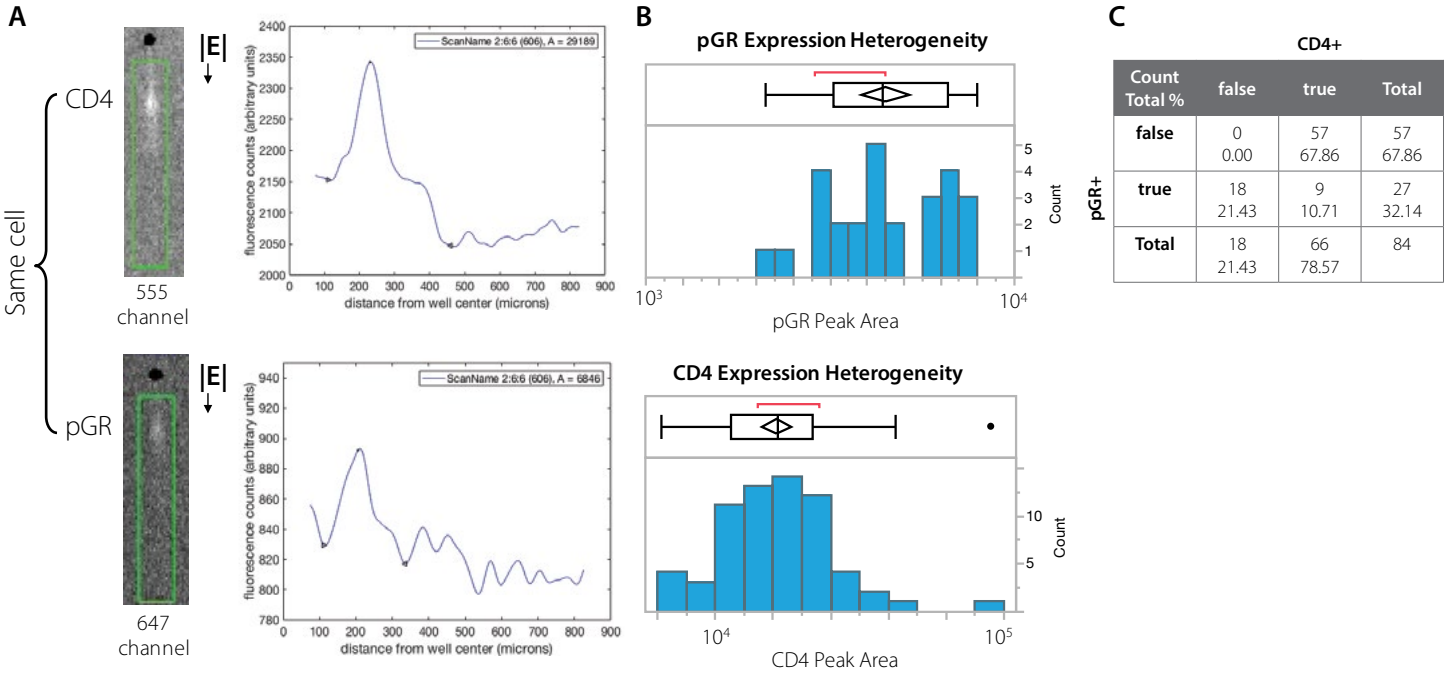


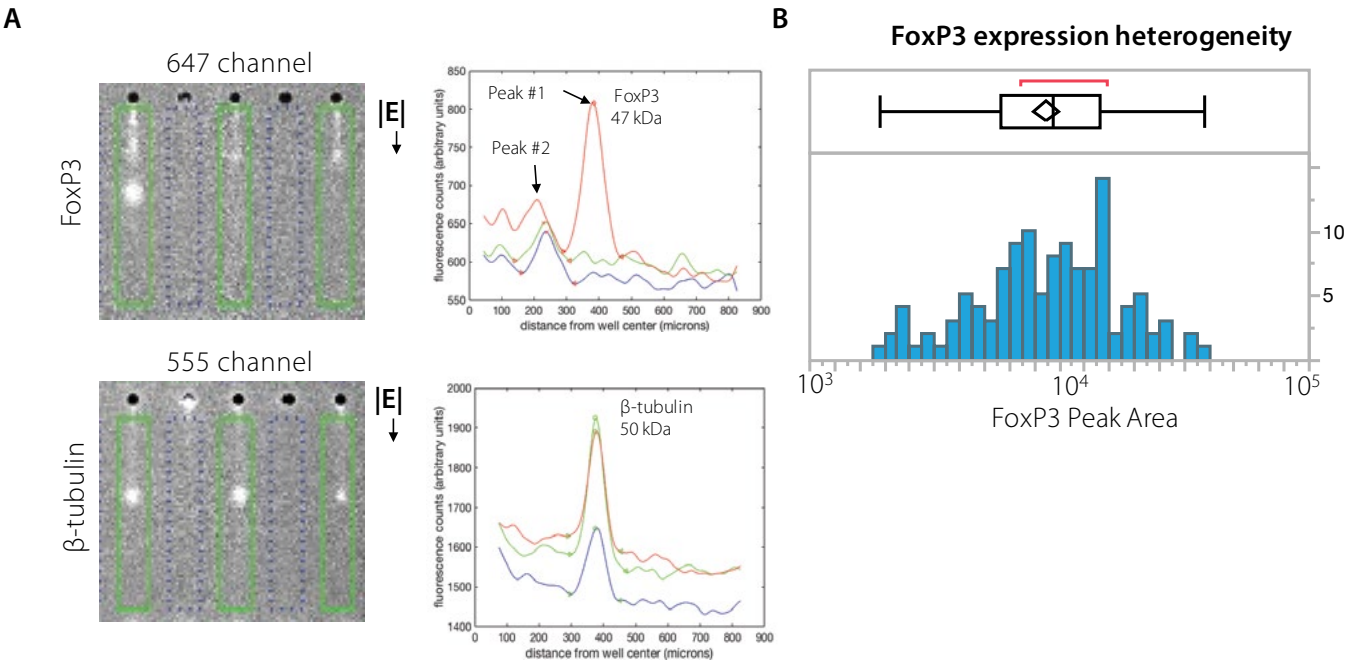
FIGURE 7. Simultaneous phospho-glucocorticoid receptor and CD4 detection in human PBMC samples. Representative separation images of pGR and CD4 and fluorescence intensity plots generated by Scout software shows clear peaks for both CD4 and pGR in the same single-cell (A). Histograms of peak areas show how CD4 and pGR expression varies across the sample (B). Analysis of cell heterogeneity for pGR and CD4 shows the number and percent of positive and negative cells in the sample (C).

Multiplexing with Single-Cell Westerns on Milo also lets you get the most data out of your precious immune samples, such as peripheral blood mononuclear cells (PBMCs). Cellular phenotypic markers and signaling proteins can be simultaneously probed to measure signaling or other effect markers within specific cellular subtypes.

Fresh human PMBC samples were analyzed using Single-Cell Westerns to measure phospho-glucocorticoid receptor (pGR), a member of the nuclear hormone receptor superfamily of transcription factors, and CD4 (**Figure 7**). GR-mediated transcriptional activation is modulated by phosphorylation and involved in cellular proliferation, inflammation and metabolism. pGR and CD4 expression heterogeneity was quantified. The fluorescence intensity plot shows clear peaks for both CD4 and pGR in the same cell (**Figure 7A**) and histograms show how expression of these two proteins varies across the sample (**Figure 7B**). Further analysis shows that 10.7% of cells were CD4+/pGR+ whereas 68% of cells were CD4+/pGR- (**Figure 7C**).

4. I have protein targets that I can't easily measure with flow cytometry

Regulatory T cells or Tregs are an important subtype of T cells that modulate the immune system and have emerged as a central target for cancer immunotherapies such as PD-L1 inhibitors. Tregs express the transcription factor FoxP3, which is commonly used to identify this cell type. However, it can be challenging to measure FoxP3 using flow cytometry because there are limited high quality flow-validated FoxP3 antibodies and the nuclear location of FoxP3 can present challenges to assay implementation. Because Milo lyses the cells and uses one streamlined workflow to measure single-cell protein expression, the standard workflow enables detection of FoxP3 in PBMCs. **Figure 8A** shows FoxP3 and β-tubulin expression in three single cells. Fluorescence intensity plots for all three single-cell separations highlighted in green show clear peaks are detected for both FoxP3 and β-tubulin. FoxP3 expression varied by almost 10-fold across the individual PBMCs analyzed (**Figure 8B**). Single-Cell Westerns on Milo also enable you to precisely enumerate the number of cells



C

FoxP3+

β-tubulin+ Count Total %	False	True	Total
False	60 4.86	24 1.94	84 6.80
True	1054 85.34	97 7.85	1151 93.20
Total	1114 90.20	121 9.80	1235

D

FoxP3 Peak #2+

FoxP3 Peak #1+ Count Total %	False	True	Total
False	984 79.68	130 10.53	1114 90.20
True	106 8.58	15 1.21	121 9.80
Total	1090 88.26	145 11.74	1235

FIGURE 8. Representative FoxP3 and β-tubulin expression from a PMBC sample. A second peak running at a higher molecular weight was also observed in cells expressing FoxP3 (A). FoxP3 expression varied by almost 10-fold across the individual PBMCs analyzed (B). Analysis of cell heterogeneity for Foxp3 and β-tubulin shows the number and percent of positive and negative cells in the sample (C). Analysis of cell heterogeneity for Foxp3 peak #1 and peak #2 shows the number and percent of positive and negative cells in the sample expressing one or the other peak (D).

that express your target of interest. In this case, 8% of the PBMCs expressed FoxP3, suggesting that 8% of the mixed sample of PMBCs were Tregs (**Figure 8C**).

This FoxP3 analysis also revealed the existence of a second peak running at a higher molecular weight (**Figure 8A**). This signal may be indicative of biologically relevant information, for example, the peak could be caused by FoxP3 dimerization, FoxP3 interaction with other regulatory proteins or it could also reflect subcellular localization of FoxP3, as Foxp3 can be found in both the cytoplasm or the nucleus. Interestingly, it appears that these two FoxP3 peaks are orthogonally expressed — 20% of cells stain positive for either Peak 1 or Peak 2 while only 1% stain positive for both peaks (**Figure 8D**). This novel observation relies on Milo's ability to resolve these two protein peaks in the molecular weight sizing separation and could not be made with flow cytometry, which relies on immune binding alone for target detection. Although Treg and other immune cell subtypes have traditionally been assayed via flow cytometry, the molecular weight sizing information provided by Single-Cell Westerns provides extra validation that the signals you are measuring can be attributed to your target of interest. The ability to measure FoxP3 and identify Tregs within clinically relevant samples such as PBMCs could be key to the development of the next generation of immunotherapies with unprecedented efficacies.

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Chapter 4

Understanding cellular complexity

Can your protein characterization platform handle the complexity of your experimental system? Are you able to accurately detect changes in cell subtype in your system? Do you struggle to distinguish protein isoforms? Don't be limited by your platform. Learn how tools from ProteinSimple can help you unravel your biology and address the fundamental questions you want answered.

What challenges do you face?

1. I'm working with mixed cellular populations

Does the heterogeneity of the tumor microenvironment challenge your protein analysis tools? You may want to analyze cell signaling in a subpopulation of tumor cells or detect target expression changes in certain cell subtypes from the immune system. Do you really know if you're seeing the whole picture when you analyze a population of mixed cells? Heterogeneity can play key roles in the tumor microenvironment and immune response. Single-Cell Westerns with Milo reveal cell subpopulations you can't see on conventional Western blots which pool thousands or millions of cells and give averaged measurements. Milo can measure protein expression in thousands of single cells in a single run so you can profile heterogeneity in your samples, and the percentage of cells in your population that are target-positive.

Fluorescent Western blots on SCO30 cell lysates using an anti-RFP primary antibody show the presence of two RFP-labeled proteins (Target 1 and Target 2, **Figure 9A**), whereas Single-Cell Western analysis of the same SCO30 cell sample reveals three distinct subpopulations of RFP expressing cells

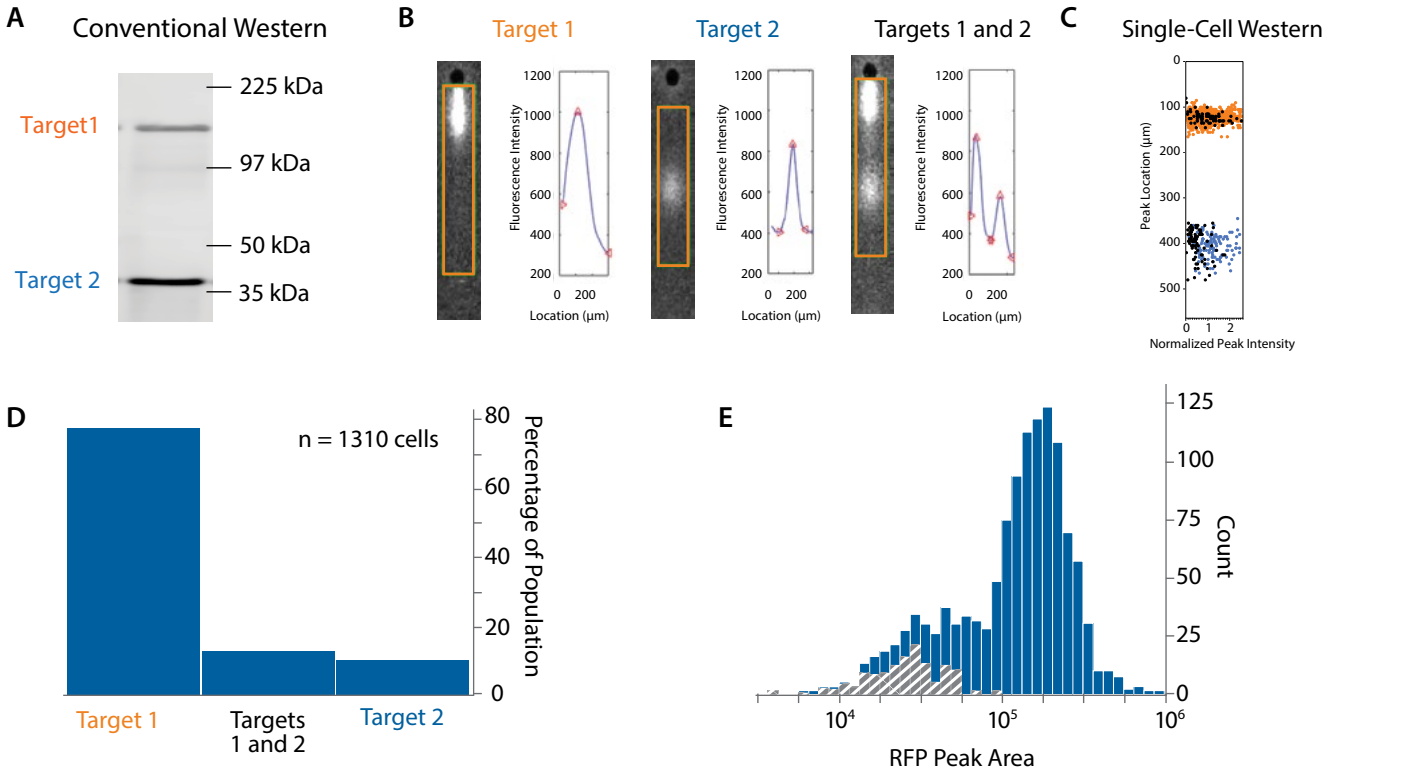

FIGURE 9. Single-Cell Westerns reveal three subpopulations of cells not visible on a conventional Western. Fluorescent Western of SCO30 cells shows two distinct RFP-labeled proteins (Target 1 and Target 2) within the bulk population (A). Single-Cell Western analysis identifies three subpopulations of cells, one expressing only Target 1, one expressing only Target 2, and one expressing both (B). Representative single-cell separation images from each subpopulation (left) and fluorescent intensity plots (right) generated by Scout software show the fluorescence intensity measured along the separation lane. Single-Cell Western data in pseudo-conventional-Western lane view (C) show cell subpopulations that contribute to the signal in each band of the conventional Western shown in (A). Percentage of cells expressing only Target 1, only Target 2 or both targets (D). The blue histogram shows the distribution of all RFP-labeled protein signal (sum of Target 1 peak area and Target 2 peak area) and represents what a flow cytometer, which integrates signal from both isoforms, would detect. The hashed gray overlaid histogram shows the distribution just of Target 2 expression across the cell population — a measurement enabled by Milo's ability to resolve species by molecular weight (E).

(**Figure 9B** and **9C**). These individual cell subpopulations in the SCO30 population express either one or both of the RFP-labeled proteins observed in the fluorescent Western and all contribute to the two bands detected. Conventional Westerns aren't able to resolve individual cell contributions to the overall signal of each band and can't detect the presence of these subpopulations. Single-Cell Westerns provide quantitative information on the percentage of cells in each of the three subpopulations. In these samples, Target 1-positive cells represent about 80% of the overall population, while Target 2-positive and Target 1/Target 2-dual-positive cells represent about 10% each of the overall population (**Figure 9D**).

2. I need to identify multiple protein isoforms or signaling pathways

Underlying our search to harness the power of the immune system to fight cancer is a need for powerful tools to dissect complex signaling pathways. Dysregulation of a single component of these complex, interconnected pathways can have potent effects on a myriad of cellular functions. Detecting multiple protein isoforms can unravel cell signaling changes in your system.

Scientists at Cedars Sinai used Single-Cell Westerns on Milo to measure protein expression heterogeneity of two distinct Blimp1 isoforms (**Figure 10**). Milo's unique molecular weight sizing step allows researchers to detect different protein isoforms that are bound by one antibody that would be unresolved in a flow cytometry measurement. Tuning the separation time on Milo enabled resolution of ~20% differences in molecular weight. Blimp-alpha (73 kDa) and Blimp-beta (92 kDa) were resolved in each single-cell separation (**Figure 10A**). Interestingly, 100% of β–tubulin+ cells were found to express Blimp1-beta while 75% of cells express both isoforms, while none of the cells express Blimp1-alpha alone (**Figure 10C**). Both isoforms varied by approximately 10-fold across the cells analyzed (**Figure 10D**). The molecular weight sizing capability of Single-Cell Westerns on Milo is key in enabling this measurement which is not possible with any other technique.

Scientists from Uppsala University used Simple Western Charge Assays on the NanoPro 1000 to develop a screen to

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"I was looking at two different isoforms of a protein, and it was important to know if the cells were expressing just one versus the other or both in a given cell. That was tricky until Milo came. We're also getting more relevant information because Milo allows us to look at expression of this protein in tissue biopsies."

— Prashant Vijay Thakkar, Ph.D., Postdoctoral Associate, Department of Medicine, Weill Cornell Medicine

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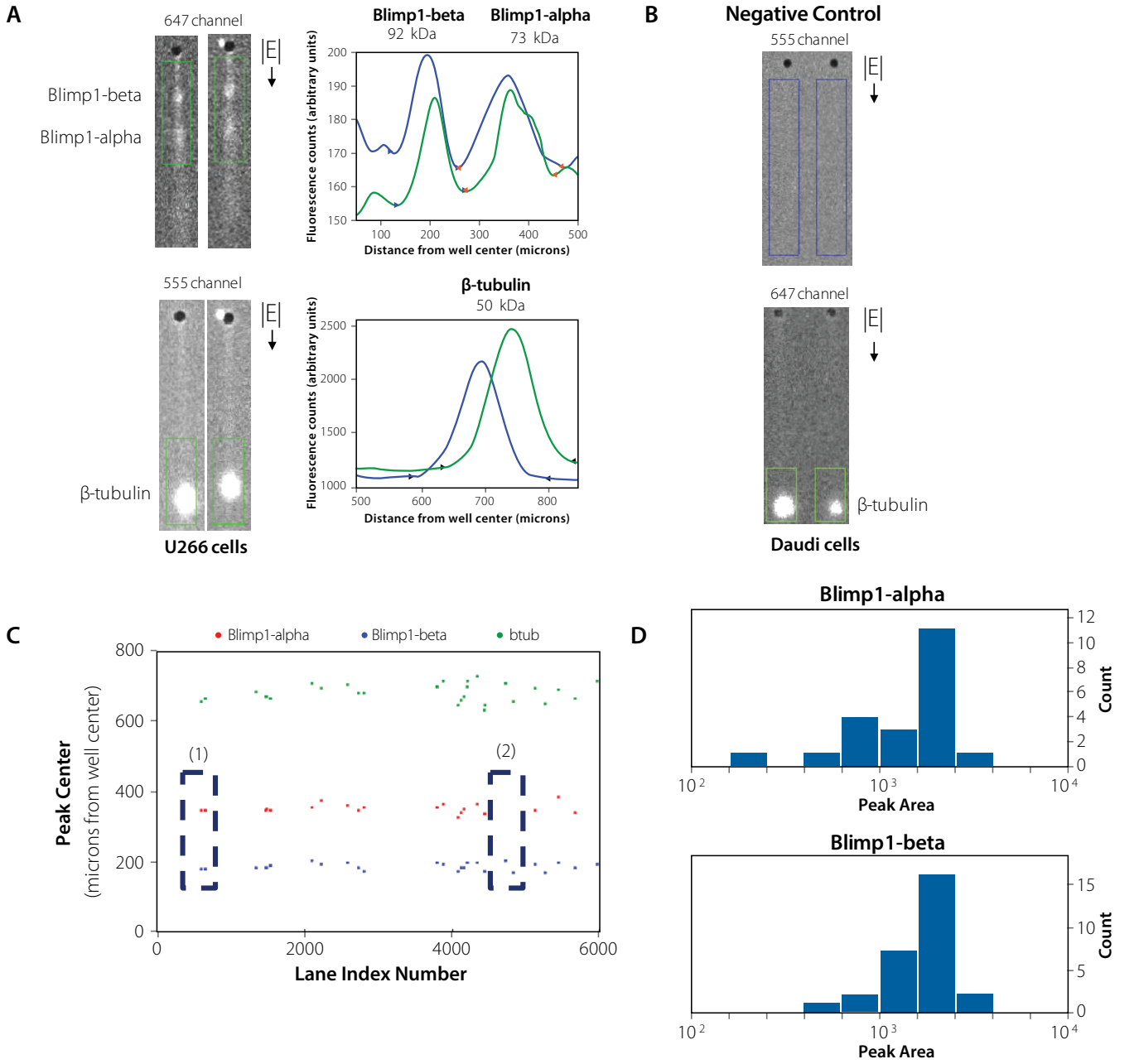


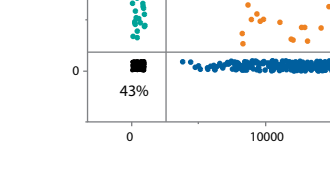
FIGURE 10. Blimp1 isoforms can be resolved by Milo. Blimp1 isoform and β-tubulin expression in single positive control (U266) cells (A) and negative control (Daudi) cells (B). Peak table showing all three protein peaks that were detected in the assay (C). The blue dots represent a higher molecular weight Blimp1 peak (Blimp1-beta), the red dots show a lower molecular weight Blimp1 peak (Blimp1-alpha) and the green dots indicate the β -tubulin peaks for each cell. Representative cells expressing both isoforms are labeled (1) while representative cells expressing only Blimp1-beta are labeled (2). Histograms of Peak Areas for the low molecular weight Blimp-1 signal (Blimp-alpha) and high molecular weight Blimp-1 signal (Blimp1-beta) show how expression of each isoform varies across the cells analyzed (D).

measure key signaling molecules in colorectal cancer with the goal of identifying complex biomarker patterns that could be used for diagnostic and prognostic purposes.[1] They found the NanoPro 1000 [assays] "…[t]o be superior to conventional immunoblotting in sensitivity and resolution... resulting in highly reproducible and sensitive detection."

In addition to being able to measure multiple signaling pathways, they were also able to identify and quantify 6 isoforms of ERK1/2 (pERK1, ppERK1, ERK1, pERK2, ppERK2, ERK2) in parallel to housekeeping proteins. Traditional Western blot required much more protein for analysis, had low reproducibility and failed to resolve phospho-variants.

To detect and quantify AKT isoforms in breast cancer, Demetris Iacovides and colleagues from Lawrence Berkeley National Laboratory used Simple Western Charge assays on the NanoPro 1000 to identify all three AKT isoforms, as well as multiple phosphorylated isoforms in breast cancer cells.[2] They were able to use this immunoassay to detect changes in AKT phosphorylation in response to pharmacological inhibitors. The sensitivity of the assay means that they can work with scarce or limited clinical samples to evaluate a patient response to drug treatments.

Jin-Qiu Chen and a scientific team from the National Cancer Institute and National Institutes of Health reviewed and evaluated Simple Western technology and concluded that it "…[o]vercomes many of the limitations of conventional proteomic approaches: it offers straightforward target-specific detection, easy operation, high quality data quantitation and excellent assay reproducibility using nanogram levels of sample" and "…[s]hows great promise for developing pathway specific diagnostics for better treatment group stratification and acceleration of drug development and disease management."[3]



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If you need to detect heterogeneity in cell signaling states within your sample, Single-Cell Westerns on Milo can simultaneously measure multiple phospho-proteins in individual cells to uncover discrete cell signaling states. In a 4-plexed experiment measuring pEGFR, total EGFR, pERK and total ERK in HCC827 cells, four discrete signaling states were observed as cells progressed through different stages in the MAPK signaling pathway (**Figure 11**). 44% of cells expressed pEGFR, one of the first proteins to become phosphorylated in the MAPK signaling pathway, but did not express any pERK which becomes phosphorylated further down the pathway. 6.1% of cells were pERK+/pEGFR+ while 6.3% of cells were pERK+/pEGFR-, suggesting that these cells exist in different signaling states. 43% of the cells were pEGFR-/pERK- and not activated. By enabling multiplexed measurement of multiple phospho-proteins, Single-Cell Westerns on Milo can reveal new insights into heterogeneity in cell signaling and response to treatment.

Single-Cell Westerns on Milo can also simultaneously measure phospho and total protein expression in the same cell to uncover the extent of phosphorylation. In unstimulated and IFNα–stimulated Jurkat cells, Milo simultaneously quantified

FIGURE 11. Milo reveals distinct cell signaling states in individual cells in a population. pERK and pEGFR expression were simultaneously quantifi ed in individual HCC827 cells. Representative images of individual single-cell lysates expressing neither pEGFR nor pERK (lane was positive for total EGFR and total ERK – not shown), pEGFR alone, pERK alone, or both pEGFR and pERK (A). Target expression for individual cells expressing pERK alone are shown in green, pEGFR alone in blue, both pERK and pEGFR in orange, and neither signal in black (individual cells positive for either total EGFR, total ERK, or both) (B).

phosphorylated pSTAT1 and total STAT1 (**Figure 12**). This simultaneous quantification reveals two sources of increased pSTAT1 expression: the number of cells expressing pSTAT1 is greatly increased in stimulated Jurkat cells (86%) versus unstimulated Jurkat cells (9%) (**Figure 12B**) and the per-cell expression levels of pSTAT1 and normalized pSTAT1 are significantly increased in stimulated Jurkat cells versus unstimulated Jurkat cells (**Figure 12C**). Single-Cell Westerns on Milo determined that the increase in overall pSTAT1 expression is attributed to an increase in both the number of cells expressing pSTAT1 and the pSTAT1 expression levels in individual cells. By quantifying pSTAT1 and total STAT1 signals in individual cells, Milo not only measures the overall percentage of cells that are phosphorylated, but also gives further detail on the extent of phosphorylation occurring within each cell (**Figure 12D**). Phospho-target quantification is often challenging or impossible using flow cytometry because of a lack of available antibodies, off-target binding, or lack of epitope availability. Single-Cell Westerns are a versatile and simple alternative to quantify phospho-target expression in single-cells.

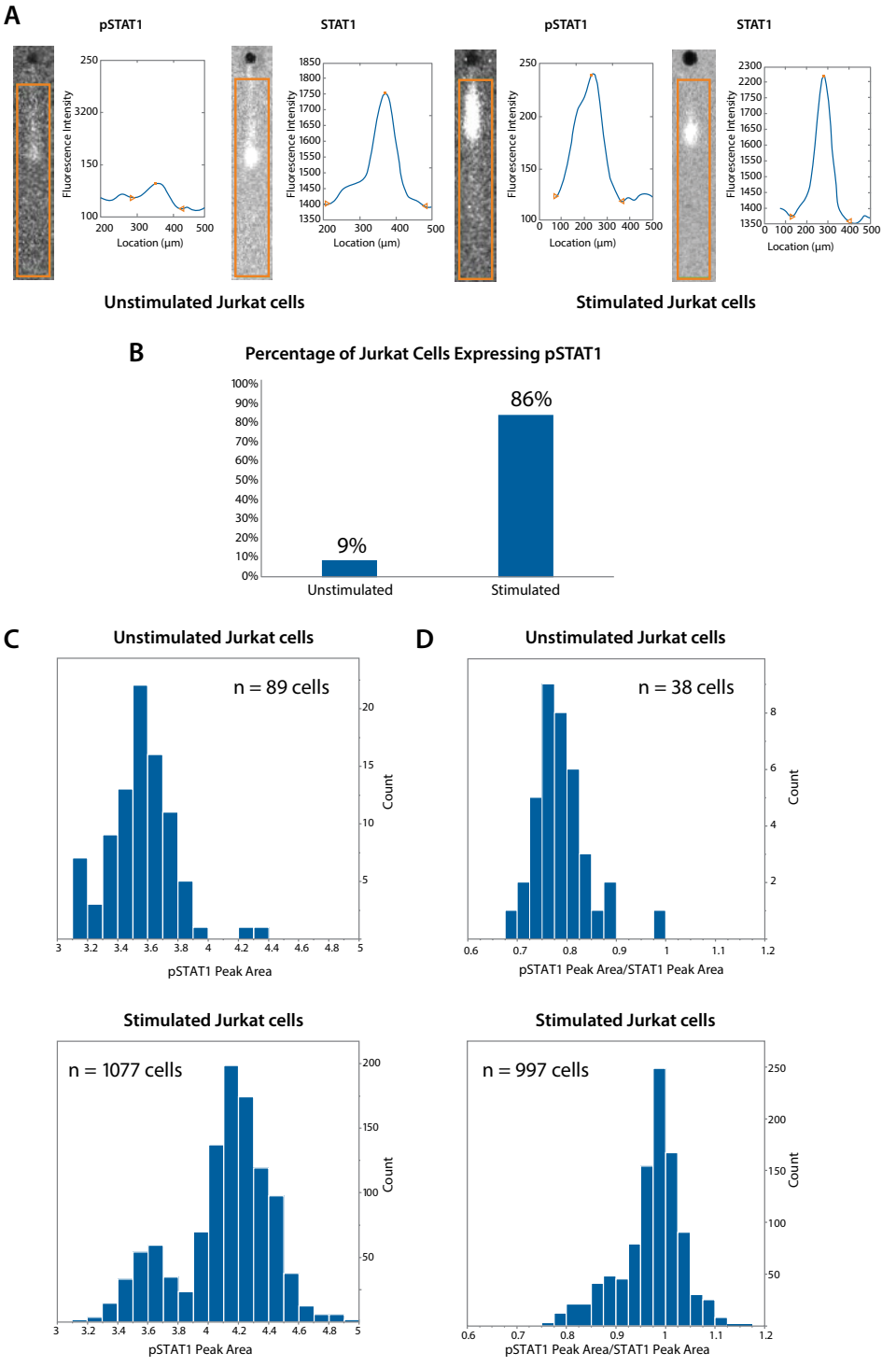

FIGURE 12. pSTAT1 and total STAT1 expression were simultaneously quantified in individual Jurkat cells either unstimulated or stimulated with IFNα. Representative lane images of individual single-cell lysates expressing pSTAT1 or STAT1 in unstimulated or stimulated Jurkat cells (A). Percentage of unstimulated and stimulated Jurkat cells expressing pSTAT1 (B). Comparison of raw pSTAT1 peak area (C) and normalized pSTAT1 peak area (D) (normalized by total STAT1 expression) in individual, unstimulated versus stimulated Jurkat cells. Stimulated Jurkat cells show significantly greater pSTAT1 peak area and normalized pSTAT1 expression.

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3. I need to measure protein modifications that are challenging to measure with other techniques

Histones are critical regulators of gene expression, and dysregulation of histone modifications is associated with oncogenesis and metastasis. Histones can be challenging to detect and quantify using traditional proteomic methods. New measurement capabilities for histones could be key to unlocking new discoveries in the field of epigenetics and the role of histone modifications in oncogenesis and metastasis. Because Milo lyses the cells before analysis and the workflow is flexible to allow for sample pre-treatments and digestions, highly complexed proteins can be measured in single cells to reveal expression heterogeneity of histone modifications. Single-Cell Westerns on Milo were able to measure heterogeneity in H3K27me3 and total histone H3 expression in single HeLa cells treated with the on-chip chromatin digestion protocol (**Figure 13**).

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Adapting the Single-Cell Western Protocol to Detect Histone Modifications Click here to download the application note

In a recent paper in Frontiers in Molecular Neuroscience, Zachary Bailey and colleagues from Virginia Tech measured histone acetylation in tissue isolated from the prefrontal cortex tissue of Sprague Dawley rats in a model of blast induced neurotrauma.[4] Using Simple Western Size Assays on Wes, they detected no changes in total histone protein expression levels, however, acetylation levels of histone

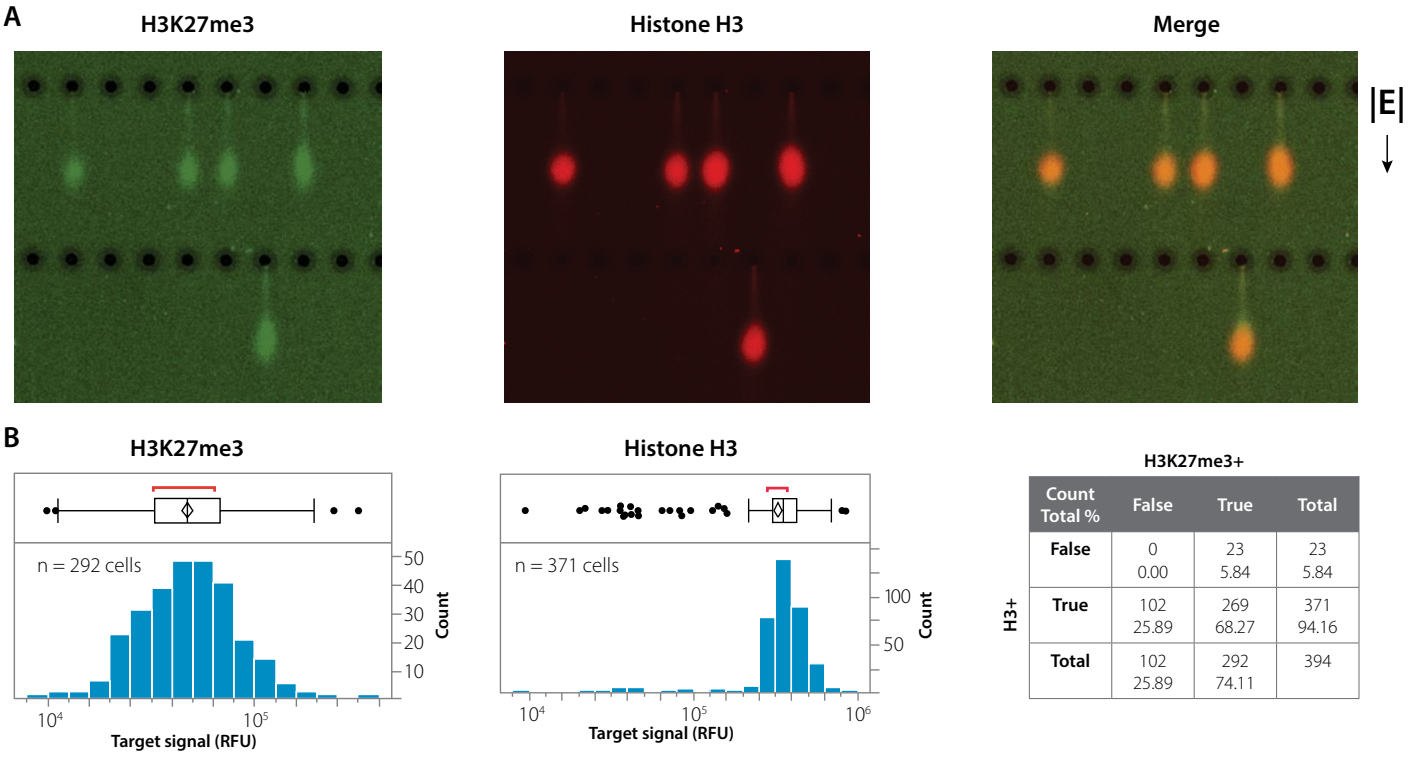

FIGURE 13. H3K27me3 signal co-localizes with total histone H3. Single-Cell Western detection of H3K27me3 (green) was validated by comigration with total histone H3 (red) (A). H3K27me3 and histone H3 signal distributions (RFU) and co-detection analysis (B). H3K27me3 was detected in ~68% of cells identified by histone H3 expression.

H2b, H3, and H4 were decreased following injury.

In a cell culture model, Melanie McConnell and colleagues from Université Paris Diderot, used Simple Western Charge Assays on NanoPro 1000 to detect acetylated and non-acetylated isoforms of the stem cell regulator PLZF in cell extracts and measure changes in levels of PLZF acetylated isoforms in the presence of HDAC3 and SIRT1.[5]

4. I need to identify protein expression heterogeneity levels

The complexity of cell types in the tumor microenvironment presents many challenges, but even when analyzing a relatively homogenous cellular population common immunoassays can't tell if some cells express very little target and some cells express a lot. Histograms generated from Single-Cell Western data on Milo (**Figure 14A**) show variation in the target expression across the population. For example, you could measure a signaling protein that is upregulated in response to a stimulus and see how variable the response to that treatment is across your population. Scatterplots (**Figure 14B**) can be used to enumerate cell subpopulations. As another example, you could probe for a protein that identifies a specific cell type within your mixed sample to see how many cells exhibit that particular phenotype. That subpopulation of cells can then be characterized by measuring expression of a second or third protein target within that subpopulation of cells.

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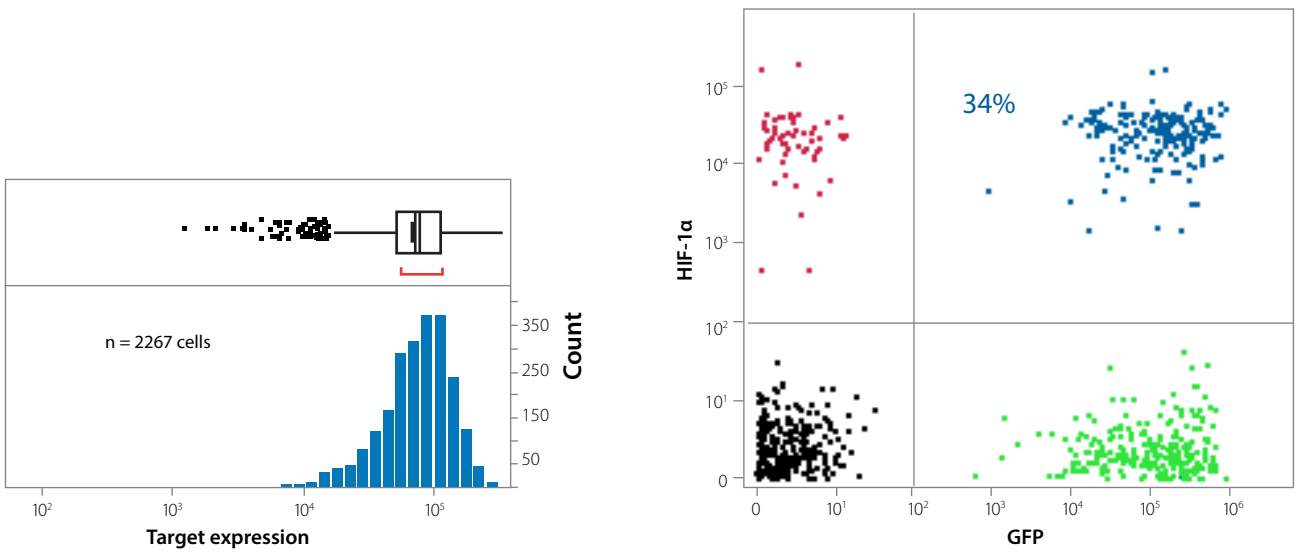

FIGURE 14. Single-Cell Westerns can quantify two types of protein heterogeneity in your sample. Histograms of peak area (A) show expression heterogeneity while scatterplots (B) show how many cells in a mixed population express each target of interest.

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Chapter 5

The need for speed: win the race to discovery

Tired of being handcuffed to your bench for hours tending to your gels and blots? Or maybe you are waiting in line for the dark room to free up? Are you tired of tedious setup methods with traditional ELISAs? Are you looking for fast method development? Your time is precious and so are your samples. You're in a race to make that next discovery, and ProteinSimple can help you get there faster.

Accomplish more

- Make new discoveries earlier
- Publish your data sooner
- Get your product to market faster

What challenges do you face?

1. I have too many samples and need higher throughput characterization

Our Simple Western assays deliver fast, quantitative protein characterization. With Wes you can process up to 25 samples in 3 hours with only 30 minutes of hands on time. If you need higher throughput, Peggy Sue, Sally Sue and NanoPro 1000 can process 96 data points overnight with only 60 minutes of hands on time. All of our Simple Western systems let you start the run, walk away, and come back to fully analyzed data.

With Ella there's no need to split your time between running assays in the lab and analyzing answers at your desk. Simply add sample and buffers to the Simple Plex cartridge, put it in Ella and start your run. There's only 10-15 minutes of hands-on assay preparation time, and fully analyzed answers are ready in an hour.

Simple Plex assays on Ella let you rapidly detect immune checkpoint molecules. Current diagnostic tests for PD-L1/B7-H1 use immunohistochemistry (IHC), which result in highly variable results due to tissue preparation, changes in detection antibodies and differing IHC cutoffs. Simple Plex offers rapid and sensitive detection for PD-L1 at low levels, from human serum/plasma, with minimal setup and results in an hour (**Figure 15**).

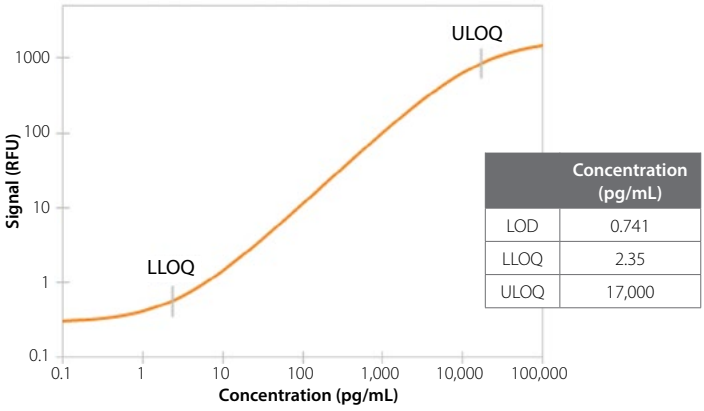

FIGURE 15. Standard curve for the PD-L1/B7-H1 Simple Plex assay on Ella. With the assay's broad dynamic range and low picogram/mL detection, low and high levels of PD-L1 are easily detected.

FROM YOUR PEERS

"We've been able to increase productivity dramatically with Wes. The results are much better too, given that it removes the blotting step all together."

— Melyssa Bratton, Ph.D., Manager of the Cell, Molecular, and Biostatistics Core, Xavier University of Louisiana

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Simple Plex Rapidly Detects the Immune Checkpoint Molecule PD-L1/B7-H1
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2. I need to reduce my drug development timelines

Imaged capillary isoelectric focusing (icIEF) assays on iCE3 give you high resolution, quantitation and automation without the limitations of other capillary IEF protein separation techniques. Charge heterogeneity analysis is complete in ~10 minutes per sample and new methods can be developed easily in a day. There's no need for product-specific methods, the same platform method can be used for multiple molecules which lets you standardize methods across product development and QC.

FROM YOUR PEERS
"Simple Plex assays on Ella gave us speed and efficiency so we could complete our assays quickly, and the high quality of the data meant that we could easily see differences between disease state samples and controls. Plus, sample preparation was so simple it reduced chances of errors significantly."

— Paulomi Aldo, Research Associate and Reproductive Sciences Core Manager, Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University

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FROM YOUR PEERS

"The short run times and easy sample preparation allowed for method development within a day or two. The versatility of the instrument allows the possibility of offering clients rapid alternatives to traditional ID and charge heterogeneity assays."

— Joan Garrison, QC Method Transfer Scientist, Cook Pharmica

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If you need to analyze mAbs, ADCs, vaccines or virus-like particles, Maurice™ gives you cIEF-based pI and charge heterogeneity data in less than 10 minutes, and size-based CE-SDS data in 35 minutes. Assay workflow is quick and simple, all you need to do is insert a ready to go cartridge, add your sample vials or a 96-well plate, and press start. You can analyze 48-100 samples per batch with CVs less than 1% and develop methods in a day.

3. I need a faster and more robust way to do my particle analysis

If you're involved in formulation testing, stability studies or final product QC, Micro-Flow Imaging™(MFI) gives you more insight into the subvisible particles present in your sample. Because MFI is image-based, you can add quantifiable morphological parameters to your analysis, allowing you to classify particle populations based on their physical characteristics. Couple one of our MFI systems with the Bot1 Autosampler to automate analysis for up to 90 samples at a time.

FROM YOUR PEERS

"As a biosimilar company, we investigate whether our products are similar to reference material, and use the MFI to perform comparability and stability. We must test samples from a variety of sources.. for all these samples, we chose the automated MFI with Bot1 because a manual-only system just wouldn't have enough throughput."

— Yuhwa Lee, Research Engineer, Samsung Bioepis

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Chapter 6

On the road to translation: product development and quality control solutions

In order to bring a biologic to market, each step of the process needs to be carefully monitored. You'll need fast and reproducible identity, purity and heterogeneity profiles for your therapeutic proteins. As you move towards formulation and final product characterization, you'll need accurate determination of subvisible particles and protein aggregates to ensure safety and efficacy of biopharmaceutical formulations due to immunogenicity concerns. Don't get lost in clinical translation! ProteinSimple can help you probe the structure and purity of protein-based therapeutics.

What challenges do you face?

1. I need a reliable platform to resolve complicated charge profiles

Most post-translational and degradation events affect the biological activity of therapeutic proteins, making charge heterogeneity analysis a critical quality attribute for molecule characterization. For fusion proteins with complicated charge variants or hydrophobic ADCs, ion exchange chromatography often suffers with poor resolution. Our Maurice and iCE3 systems employ ProteinSimple's icIEF technology, iCE, which significantly improves resolution with a reproducible peak pattern with a run time of only 10 minutes.

John Valliere-Douglass and colleagues from Seattle Genetics use iCE to monitor covalent modifications of lyophilized, heat-stressed formulations of mAbs and ADCs.[1] Determining the effect of these covalent modifications is critical to establish appropriate storage conditions as they can contribute to protein heterogeneity. They found icIEF "…[t]o be quite sensitive for assessing covalent adduction resulting from condensation-type reactions", with an advantage "of separating molecule charge variant populations on the basis of the pI of the entire molecule", compared to chromatographic techniques which separate charge variants on the basis of exposed charge.

Concerned about transferring methods to different labs? A study conducted across 12 laboratories from 11 independent biopharmaceutical companies evaluated icIEF for the charge heterogeneity analysis of monoclonal antibodies.[2] They concluded that icIEF is a robust and reliable technology that supports "the application of icIEF methodology both in process development and quality control of biopharmaceutical companies."

Maurice's native fluorescence detection for icIEF assays works by measuring the fluorescence emission of tryptophan's aromatic group. It's also label free so you don't have to optimize protein labeling or run into issues with background noise when the label unconjugates from your protein. Baselines are significantly cleaner and less sensitive to ampholyte interference, giving you more options when optimizing your pH gradient. You'll also get 3–5X more sensitivity compared to UV absorption. That means sample concentration and desalting isn't needed, which decreases sample preparation time. Because proteins tend to aggregate less at lower concentrations, you'll also be able to reduce or even remove urea completely in some of your methods.

To show how versatile Maurice's fluorescence mode is, we tested three different types of proteins with different pIs, isoform profiles, and molecular weight. Separation conditions for a 70 kDa therapeutic protein, 200 kDa fusion protein, and 150 kDa monoclonal antibody were optimized for resolution and signal for each molecule (**Figure 16**). All proteins had intrinsic fluorescent properties that generated high resolution profiles, confirming there is no bias when it comes to the type of protein you can run on Maurice. Any protein that contains tryptophan residues can be analyzed on Maurice in fluorescence mode with an ampholyte mixture that delivers the required resolution.

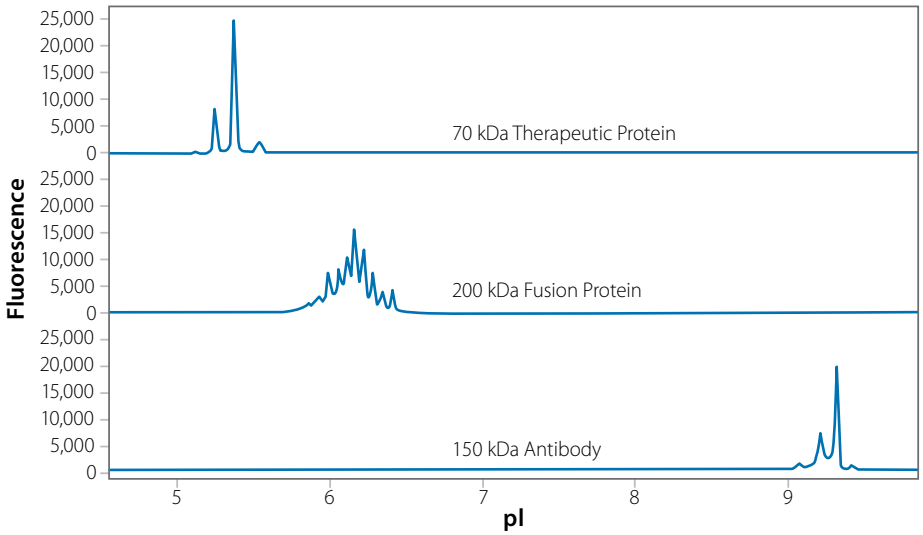

FIGURE 16. A therapeutic protein, fusion protein, and a monoclonal antibody were run on Maurice and detected in fluorescence mode. 200 µg/mL of therapeutic protein and 250 µg/mL of mAb were run with 6% 3-10 Pharmalyte, 10 mM arginine, 10 mM iminodiacetic acid, and 2M urea, then separated for 1 minute at 1500 V followed by 7 minutes at 3000 V. 400 µg/mL of fusion protein was run with 4% 5-8 Pharmalyte/2% 3-10 Pharmalyte, 10 mM arginine, 10 mM iminodiacetic acid and focused for 1 minute at 1500 V followed by 8 min at 3000 V. The fluorescence exposure time was 10 seconds for the fusion protein and mAb, and 5 seconds for the therapeutic protein.

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Improving Charge Variant Analysis with Maurice Native Fluorescence Click here to read the application note

2. I need to know more about the particles in my sample

Accurate determination of subvisible particles and protein aggregates is important to ensure the safety and efficacy of biopharmaceutical formulations due to immunogenicity concerns. Biopharmaceutical manufacturers are expected to characterize, monitor and control subvisible protein particles and non-protein particles in their products. Accurately and precisely characterizing particle populations for a product is increasingly important as regulatory agencies require this as proof of a robust and reproducible manufacturing capability. This information helps establish comparability between lots of drugs as well as biosimilars and their originator molecules.

As traditional techniques such as light obscuration lack the sensitivity to distinguish translucent and potentially harmful protein aggregates, other options are needed. MFI offers several advantages over traditional techniques in the analysis of subvisible and visible particles in protein formulations as its image-based approach offers direct particle detection. The particle count, size and morphological information provided enables novel and unique insights into particle characterization and quantification with just a single test. As a result, MFI systems can discriminate protein aggregates from the silicone micro-drops, air bubbles and other contaminants commonly found in complex biopharmaceutical samples.

LEARN MORE
Confirming Accurate Particle Counting and Sizing on MFI Systems Click here to read the application note

FROM YOUR PEERS

"MFI technology has allowed us to better understand what conditions promote stability of our therapeutic molecules, which ensures that only world-class medicines are delivered to patients."

— Stephanie Davies, Ph.D., Formulation Sciences, MedImmune

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3. I need to be 21 CFR Part 11-compliant

Biotherapeutics, including immuno-oncology drugs, are manufactured under strict regulations to ensure their quality. Electronic data authenticity and integrity are an integral part of GMP manufacturing for pharmaceutical companies, and all analytical instruments must be compliant with the FDA Title 21 Code of Federal Regulations (CFR) Part 11. iCE3, Maurice and MFI systems all support compliance with 21CFR Part 11 in QC and GMP environments

References

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2. Robustness of iCIEF methodology for the analysis of monoclonal antibodies: an interlaboratory study. O Salas-Solano, B Kennel, SS Park, K Roby, Z Sosic, B Boumajny, S Free, A Reed-Bogan, D Michels, W McElroy, P Bonasia, M Hong, X He, M Ruesch, F Moffatt, S Kiessig, B Nunnally. *J Sep Sci*, 2012; 35:3124–9.

LEARN MORE

Staying 21 CFR Part 11-compliant with Maurice and Compass for iCE Click here to read the application note

Compass for iCE (V2.0) Title 21 Code of Federal Regulations (CFR) Part 11 Compliance Checklist Click here to read the application note

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