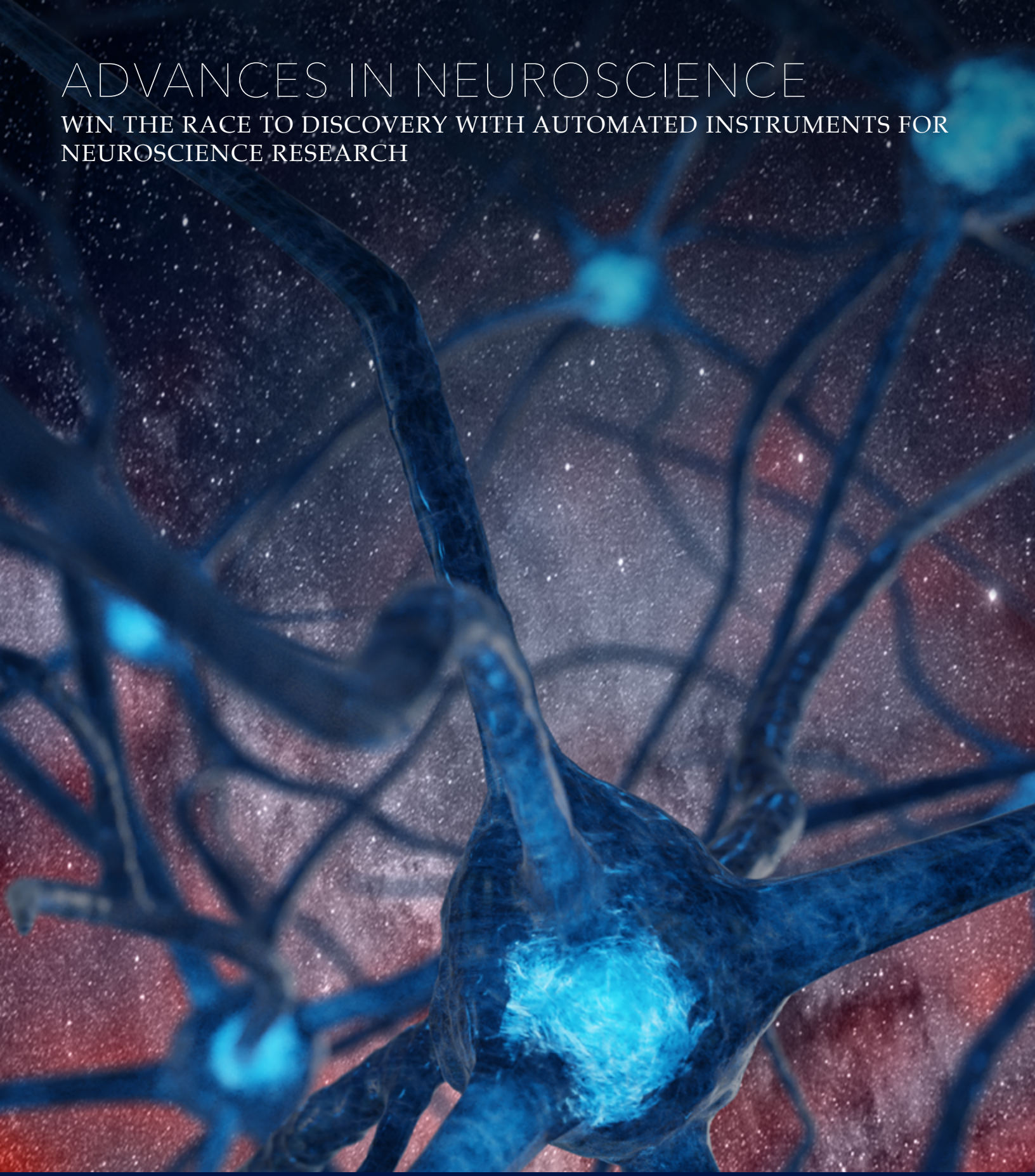
ADVANCES IN NEUROSCIENCE

WIN THE RACE TO DISCOVERY WITH AUTOMATED INSTRUMENTS FOR NEUROSCIENCE RESEARCH

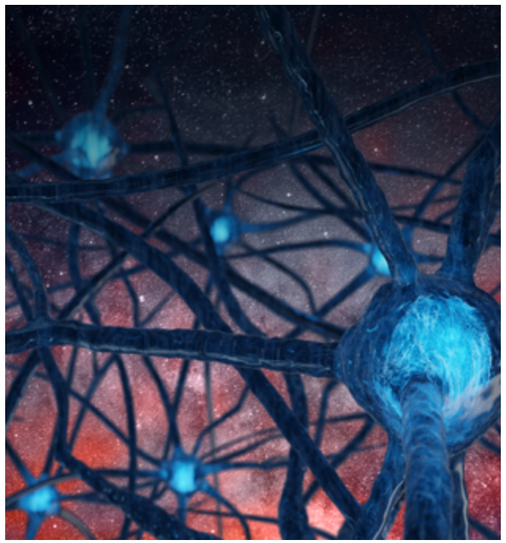


bio-techne®

NEXT GENERATION PROTEIN ANALYTICAL SOLUTIONS FOR NEUROSCIENCE

Neurological disorders are the number one cause of disability globally. Odds are that you know someone affected by a neurological disorder, as The World Health Organization estimates that one billion people are affected by them, contributing to 11% of the world's disease burden. Advanced protein analytical tools are needed to address grand challenges in neurological disorders. For this reason, Bio-Techne offers Simple Western™, Single-Cell Western with Milo™, Simple Plex™, and FluorChem™ Imagers that detect and analyze proteins from small volumes of sample at unprecedented speeds and sensitivity.

Bio-Techne enables scientists to explore new facets of neurological disorders with hard-to-get and often very small patient samples. Many scientists count on Simple Western, Single-Cell Western, Simple Plex, and FluorChem as essential tools for the discovery and validation of biomarkers for conditions such as Parkinson's disease, traumatic brain injury, dementia, Alzheimer's disease, multiple sclerosis, and epilepsy. This eBook will guide you through some of the most predominant neurological disorders to show how Bio-Techne instruments have contributed to research and discovery within these specific disease areas.



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CHAPTER 1: FULLY AUTOMATED WESTERN ANALYSIS WITH SIMPLE WESTERN

SAVE YOUR SAMPLES AND YOUR TIME WITH SIMPLE WESTERN

Traditional Western blots are challenging and fraught with limitations that hold back advancements in neuroscience. Traditional Western blots are time-consuming, poorly reproducible, low throughput, and hardly quantitative. Even worse, Western blots require large amounts of precious neruological sample that makes detection of low abundance proteins a challenge. As a result, researchers often have to pool precious samples like brain tissue together, which can interfere with statistical analysis.

In challenging situations like these, Simple Western can make a world of difference. Simple Western is a fully automated, capillary-based immunoassay platform that generates Western blot data on up to 24 samples in as little as 3 hours. With Simple Western, researchers only need 3 µL of precious sample to get multiple data points covering a molecular weight range from 2 to 440 kDa. Simple Western is fully automated and with that comes reproducibility reflected in intra-assay CV <15%. For those interested in investigating charge isoforms, our Simple Western Charge Assay gives you all the detailed info you need including characterization of post-translational protein modifications of up to 96 samples at a time. Even better, this all comes without ever having to run a gel or touch a blot.

- Click Here to Learn More about Simple Western

Click Here to View a Quick Simple Western Video

STOP POOLING MICE TO GET WESTERN BLOTTING DATA

Because Simple Western requires only 3 µL of sample while offering pg-level sensitivity, there is no need to pool samples like mouse tissue and other samples. Not only does Simple Western eliminate the interference that pooling samples can have on statistical data, Simple Western provides significant savings on cost and mouse lives. Simple Western also ups the number of data points you can obtain from laser capture microdissection samples.

The high throughput that Simple Western provides, along with fully quantitative data, allow for the creation of accurate dose-response curves. Simple Western can analyze up to 96 samples overnight without user intervention, meaning a dose-response curve can be generated in a fraction of the time it would take with traditional Western blot.

MULTIPLEX DETECTION GETS MORE DATA PER SAMPLE

To get the most data out of precious samples, Simple Western offers RePlex™, which efficiently removes the antibodies from the first round of probing for a second round of probing with fresh antibodies. The second round may also be used for Total Protein Detection, so you can normalize your data with confidence. To multiplex even further, Simple Western with Jess™ offers multi-color Western analysis with chemiluminescence and fluorescence (IR/NIR) detection channels (**FIGURE 1**).

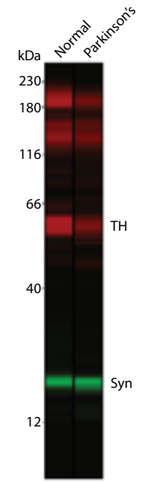

FIGURE 1. Using Jess to confirm downregulation of tyrosine hydroxylase (TH) in Parkinson's disease. With Jess, multiplexing another neuronal marker, in this case, synuclein (Syn), is easy and provides a more meaningful picture of the biology.

THE SIMPLE WESTERN SIZE ASSAY QUANTIFIES PROTEIN EXPRESSION LEVELS IN HUMAN BRAIN SAMPLES WITH SUPERIOR SENSITIVITY TO WESTERN BLOTTING

P-glycoprotein (P-gp) is linked to Aβ clearance and has important implications for Alzheimer's disease pharmacology, epidemiology and genetics. Here, Simple Western was used to measure P-gp expression in human brain capillaries of patients with Alzheimer's disease. Using the sensitive and quantitative nature of Simple Western, they demonstrated a 30% reduction in P-gp levels in patients with Alzheimer's disease (**FIGURE 2**). The sensitivity of Simple Western enabled reproducible quantifications of P-gp at the nanogram level.

In the paper, the authors noted the superior sensitivity and reproducibility of Simple Western:

"This novel assay allows protein quantitation at 10-fold higher sensitivity and better reproducibility compared to Western blotting"

- Click Here to Download this Paper
- Click Here to Learn More about the Simple Western Size Assay

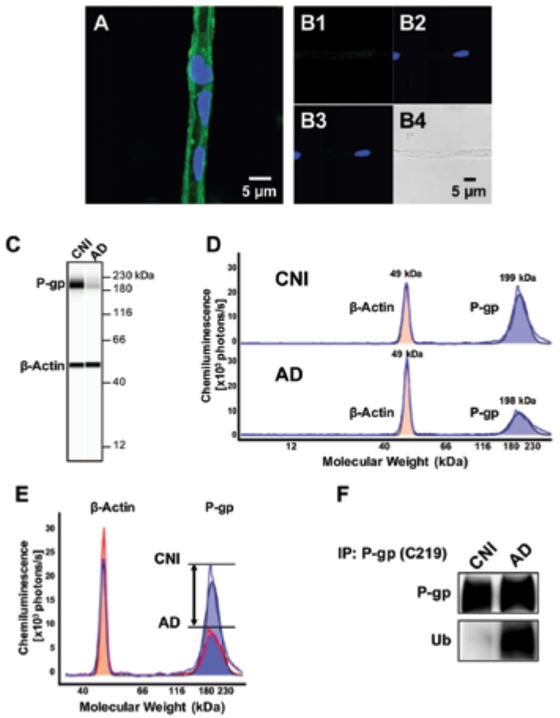

FIGURE 2. P-gp expression levels are decreased and P-gp ubiquitination levels are increased in brain capillaries from Alzheimer's disease (AD) patients. (A) Representative image of a P-gp-immunostained (green) brain capillary isolated from brain tissue of a cognitive normal individual (CNI); nuclei were counterstained with DAPI (blue). (B) Negative control (no primary antibody): (B1; green channel); (B2; blue channel); (B3; overlay of green and blue channel) and (B4; transmitted light channel). (C) Representative Wes lane view image and (D) electropherograms showing reduced P-gp (blue shaded area) protein expression levels in brain capillaries isolated from human brain tissue (frontal cortex) of AD patients (n = 3) vs. CNIs (n = 3). (E) Overlay of electropherograms displayed in (D) shows a reduction in the area under the curve (AUC) that represents P-gp protein expression levels (blue shaded area) in brain capillaries from AD patients (red line) relative to CNI (blue line). In contrast, β-actin levels (orange shaded area) in brain capillaries from AD patients (red line) and CNI (blue line) were the same. (F) Traditional Western blot showing that ubiquitin levels in P-gp-immunoprecipitates are increased in capillaries from AD patients compared to those from CNI. Adapted with permission from Hartz *et al*. (2018) *Frontiers in Aging Neuroscience* (CC BY 4.0)

THE SIMPLE WESTERN CHARGE ASSAY AS A NOVEL TOOL TO PREDICT RISK OF PARKINSON'S DISEASE DEMENTIA: A CLINICAL STUDY.

Since early detection of dementia in Parkinson's disease is a prerequisite for preventive therapeutic approaches, efforts have been made to identify markers that will assist in its detection. One such market is serpinA1 in cerebrospinal fluid (CSF), which was suggested as an early biomarker for differentiation between Parkinson's patients with (PDD) or without dementia (PD). Earlier studies using 2D-PAGE had shown differentially sialylated SerpinA1 isoforms in PDD patients compared to control and PD patients. However, 2D-PAGE is time-consuming and cannot be used as a high-throughput approach. CSF as a sample type is challenging to obtain, motivating the desire to use as little sample as possible. Furthermore, CSF often contains low protein loads, making sensitivity an important consideration for protein assays.

To further explore the diagnostic value of serpinA1, the researchers of this study applied a newly developed method for the detection of serpinA1 based on the Simple Western Charge Assay on clinical samples from 102 subjects including neurologically healthy controls (CON), PD and PDD patients (**FIGURE 3**).

The authors conclude that the novel serpinA1 Simple Western Charge Assay can help to discriminate PDD patients from control and PD patients in a standardized, fast, and high-throughput compatible way. Moreover, the CSF serpinA1 isoform analysis might already predict cognitive impairment in PD patients who will develop dementia in the course of the disease, as they found that patients with a positive serpinA1 test have more than 6 times higher risk of an association with dementia. The measurement of serpinA1 can therefore support the early clinical diagnosis of dementia in PD patients and help to stratify patient populations for therapeutic trials.

- Click Here to Download this Paper
- Click Here to Learn about the Simple Western Charge Assay

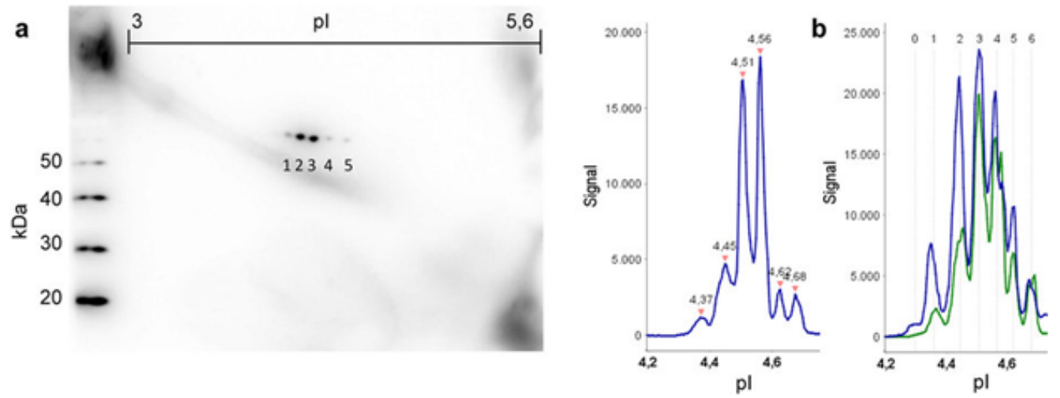

FIGURE 3. (**a**) Comparison of a control 2D serpinA1 immunoblot with the corresponding CIEF electropherogram. The peaks at 4.51 and 4.56 match the two intense spots from the 2D immunoblot. The two more basic and one more acidic isoform can also be found in the immunoblot. However, the most acidic peak in the electropherogram cannot be seen in the 2D immunoblot. (**b**) Overlay of one control (green) and one PDD (blue) electropherogram. The difference in the relative abundances especially of the more acidic isoforms can clearly be seen. The isoforms corresponding to peak 1 and peak 2 are noticeably increased in the PDD sample as compared to controls. Peak 0 is only detectable in the PDD patient. Signal intensity in chemiluminescence units; pI, isoelectric point. 2D exposure time 2 s. CIEF exposure time 30 s. Adapted with permission from Halbgebauer *et al*. (2016) *Nature Scientific Reports*. (CY BY 4.0)

CHAPTER 2: SINGLE-CELL WESTERNS WITH MILO

MEASURE PROTEINS IN INDIVIDUAL NEURAL CELLS WITH SINGLE-CELL WESTERNS ON MILO

Milo™ is the world's first automated single-cell western (scWestern) platform. The instrument measures protein expression in thousands of cells in a single run, allowing you to profile heterogeneity in your samples through single-cell analysis. 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. Then just probe with your favorite conventional western blot antibodies to measure ~12 proteins per cell using a variety of multiplexing strategies. Our Single-Cell Western technology on Milo unlocks the single-cell proteome to measure more of the proteome than was possible with any other single-cell protein analysis technique.

- Click Here to Learn More about Milo

Click Here to View a Quick Milo Video

The Milo™ Single-Cell Western platform allows you to identify and quantify the percentage of neural subtypes in your sample and track differentiation progression based on subtype-specific marker expression analysis. We have demonstrated how Milo can be used to identify and quantify neural subtypes in a heterogeneous neural sample, and monitor the differentiation of induced pluripotent stem cells (iPSCs) into neurons, astrocytes, and oligodendrocytes using R&D Systems research-grade or GMP differentiation reagents.

- Click Here to Download Our Application Note: Identify and Quantify Neural Subtypes with Single-Cell Westerns

The Milo Single-Cell Western system can analyze multiplexed protein expression patterns in dissociated tissues to identify and quantify cell subtypes contained in heterogeneous tissues. In this application note, we describe and characterize a protocol to successfully dissociate mouse neural tissue microsurgically dissected from combined cortex, ventricular zone, and hippocampus regions of E18 mice into single cells. Using a multiplexed Single-Cell Western assay to analyze protein expression in the dissociated single-cells, we identify 3 subpopulations of cells within the tissue: astrocytes, radial glia, and immature neurons based on expression of their respective phenotypic protein markers: GFAP, Pax6, and β-III tubulin (**FIGURE 4**). Cell subpopulations that are single- or multi-positive for each marker are quantified to demonstrate how cell subtypes can be identified and quantified based on multiplexed protein expression patterns using Single-Cell Western technology.

- Click Here to Download our Application Note: Dissociation of Mouse Neural Tissue for Single-Cell Western Analysis

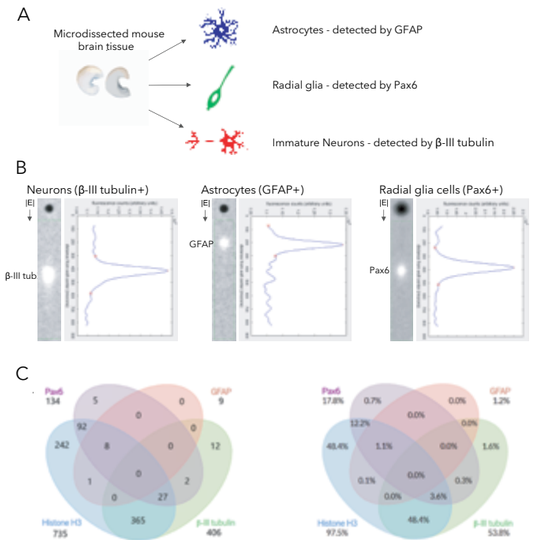

FIGURE 4. (**A**) E18 mouse combined cortex, ventricular zone, hippocampus tissue composition. Immature neurons constitute the largest percentage of cells while astrocytes and radial glia are also present. (**B**) Milo identifies neurons (β-III tubulin+), astrocytes (GFAP+), and radial glia (Pax6+) in dissociated neural tissue. Separation images and fluorescence intensity plots show clearly detected protein bands for each target. (**C**) Multiplexed 4-target Single-Cell Western assay identifies cell subpopulations in dissociated primary neural tissues. Venn diagrams reveal number (left) and percentages (right) of single- and multi-target positive subpopulations. Measured percentage of β-III tubulin+ cells (53.8%) matches expected neuron percentage of 50% as measured by manufacturer (BrainBits).

CHAPTER 1 CHAPTER 2 CHAPTER 3 CHAPTER 4

GUTS AND GLORY: VALIDATING A NEUROEPITHELIAL CIRCUIT USING MILO

"We have been pairing Milo with single-cell real-time qPCR data. We have the RNA and we know the transcripts are present. We used Milo to confirm our results and quantify the amount of protein that is in each individual cell."

- M. Maya Kaelberer, Ph.D., Postdoctoral Associate, Duke University School of Medicine

FOOD FOR THOUGHT

Melanie Maya Kaelberer, Ph.D., is a Postdoctoral Associate at Duke University whose research has already significantly contributed to a rapidly emerging area of interest: gut-brain signaling. Dr. Kaelberer is part of a Duke research team studying how a chain of synapses converts food to thought. They've adopted Milo™ to help unveil the first synapse right at the gut surface—where it first meets the brain.

HOW DOES THE GUT TALK TO THE BRAIN?

As it turns out, a newly identified population of gut sensory cells they call "neuropod cells" harbor the previously unknown answer to the question of how the gut can rapidly communicate with cranial nerves. Using a combination of techniques and tools, Dr. Kaelberer and colleagues are uncovering the role of neuropod cells to synaptically convey information onto neurons, which enables the brain to sense gut stimuli.

In need of a proteomic, single-cell approach that could validate the high-resolution, pre-synaptic and transcriptomic information they had obtained with other methods, the team turned to Single-Cell Westerns on Milo and showed that a subset of enteroendocrine cells contains presynaptic adhesion proteins, including some necessary for synaptic adhesion. "We used Milo to identify how many gut sensory cells are neuropod cells and thereby contain the pre-synaptic protein Synapsin-1, enabling them to transduce stimuli onto neurons," clarifies Dr. Kaelberer. The cells contain presynaptic adhesion proteins, including some necessary for synaptic adhesion. "We used Milo to identify how many gut sensory cells are neuropod cells and thereby contain the pre-synaptic protein Synapsin-1, enabling them to transduce stimuli onto neurons," clarifies Dr. Kaelberer.

MILO AND THE IMPORTANCE OF VALIDATION

Single-Cell RNA studies allow researchers to analyze RNA expression heterogeneity in individual cells in a single assay, but cellular protein levels aren't always directly proportional to mRNA concentration. This makes the validation of single-cell RNA expression data on a protein level especially important to ensure accurate and complete conclusions are made about cellular function. Milo made single-cell resolution Western blotting possible for researchers at Duke.

Using neuropod cell samples, Dr. Kaelberer says, "Milo allowed us to quantify the amount of protein at a single-cell level and show that neuropod cells have the machinery to form synapses." She continues, "In research, it is important to show results using different methods. We had single-cell RNA and immunohistochemistry data, and we used Milo to confirm our results." Using Milo to supplement their results, Dr. Kaelberer and colleagues' research efforts recently culminated in a high-profile *Science* publication.

When asked if there's anything else she'd like to add, Dr. Kaelberer took the opportunity to highlight the usability of Milo's accompanying Scout™ software, "The analysis software makes data analysis very straightforward." Indeed, Scout software automates quantitative data analysis. The overall Single-Cell Western workflow is flexible and can be tailored to your protein target and samples of interest.

- Click Here to Download the Paper

CHAPTER 3: NEXT GENERATION ELISAS WITH SIMPLE PLEX ON ELLA

NEUROSCIENCE BIOMARKER DISCOVERY AND VALIDATION WITH SIMPLE PLEX™ ASSAYS ON THE ELLA™ PLATFORM

Standard ELISA techniques for detecting protein biomarkers in serum or other biological samples often lack sensitivity and don't quite make the grade for evaluating many neurological disorders. Many scientists have become frustrated with assays that offer low reproducibility and tedious manual workflow with hours of hands-on time. Because each manual step introduces variability, the only way to rule out human error is to run samples in duplicate, which consumes precious sample volume. These drawbacks have hindered the adoption of the ELISA for investigating complex, multivariate diseases like neurodegenerative diseases and traumatic brain injury.

Ella transforms the way you perform an ELISA by offering a fast and robust method for single or multi-analyte testing. By automating the immunoassay, Simple Plex assays on Ella eliminate traditional ELISA challenges, giving you more precise data without all the hassle in 90 minutes.

It all happens on disposable Simple Plex cartridges, which come in four flavors: single-analyte cartridges that let you analyze up to 72 samples, multi-analyte cartridges that let you analyze up to four analytes in 32 samples, multiplex cartridges that let you analyze up to eight analytes in 32 samples, and customizable digoxygenin cartridges that let you analyze up to 48 samples utilizing your own reagents. With Ella, you'll be able to achieve reproducible sensitivity – will as little as 2.5 µl of your sample!

- Learn More about Ella and Request a Quote
- Choose the Best Simple Plex Assay for your Needs

Click Here to View a Quick Ella Video

THE AUTOMATED SIMPLE PLEX WORKFLOW

1. Load sample, start the assay, and walk away
2. The sample flows through microfluidic channels containing glass nanoreactors (GNRs)
3. Antibody coated GNRs capture target analyte
4. Stringent wash removes any unbound sample
5. Detection antibody flows through the GNR
6. Stringent wash removes unbound detection antibody
7. A Streptavidin-conjugated fluorescent dye flows through the GNRs
8. Stringent wash removes unbound fluorescent dye
9. Scan GNRs

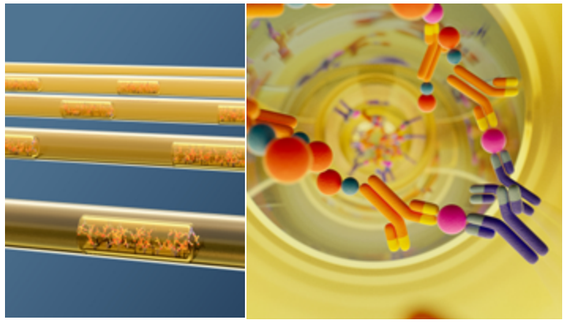

FIGURE 5. The automated, sample-sipping Simple Plex assay features built-in replicate measurements and quantifies up to 8 analytes from as little as 2.5ul of your sample. The image on the left depicts microfluidic channels containing glass nanoreactors (GNRs). The image on the right depicts immunoassay sandwiches inside a GNR.

CHAPTER 1 CHAPTER 2 CHAPTER 3 CHAPTER 4

SIMPLE PLEX NF-L ASSAYS ARE SENSITIVE AND COST-EFFECTIVE

Neurofilament light (NF-L) protein is elevated in cerebrospinal fluid (CSF), serum, and plasma following damage to the neuronal cytoskeleton. This is a common feature of neurodegenerative diseases such as Multiple Sclerosis (MS) and Amyotrophic Lateral Sclerosis (ALS). As such, it represents a promising liquid biomarker for investigators. Gauthier *et al*., (2021) recently ran a side-by-side comparison between the Simple Plex NF-L assays and the established Simoa Human Neurology 4-Plex "A" Kit. MS sample data generated using the Simple Plex assay was concordant with data generated using the Simoa assay. Assay sensitivity was excellent for both assays. The authors conclude that the Simple Plex NF-L assay is a reasonable and cost-effective alternative, particularly for routine NF-L quantification. Finally, our ALS data was consistent with that of Gauthier *et al*., (2021). Data generated using Simple Plex immunoassays were robust and highly correlated with Simoa assays and Uman ELISAs.

Why should you choose Ella? Simple Plex automated assays get you data in 90 minutes or less, with minimal opportunity for user error. The Ella platform has a small footprint. Simple Plex assays are relatively cost-effective, allowing you to allocate your resources most effectively. Finally, Simple Plex assays are sensitive, detecting picogram per milliliter NF-L concentrations in CSF, serum, or plasma, allowing you to conserve your precious sample.

- See How Simple Plex Assays Quantify NF-L in Multiple Sclerosis
- See How Simple Plex Assays Quantify NF-L in Amyotrophic Lateral Sclerosis
- Learn More About the Simple Plex NF-L assay
- Purchase the Simple Plex NF-L assay

SIMPLE PLEX NF-H ASSAYS EXPEDITE SPINAL MUSCULAR ATROPHY BIOMARKER DISCOVERY AND TESTING

Spinal Muscular Atrophy (SMA) is an autosomal recessive neurodegenerative disease characterized by degeneration of alpha motor neurons and muscle weakness. Predictive and prognostic biomarkers are urgently needed to combat this disease. Elevated blood and CSF levels of Neurofilament heavy (NF-H) have been reported in Amyotrophic Lateral Sclerosis, Alzheimer's disease, Multiple Sclerosis, and Parkinson's disease. Darras *et al*., (2019) evaluated NF-H's role as a biomarker for SMA. Using the Simple Plex, phosphorylated NF-H (pNF-H) assay, the investigators quantified NF-H in EDTA plasma from children with or without SMA. Infantile-onset SMA was correlated with ~10 fold higher pNF-H level than age-matched infants, and ~90 fold higher pNF-H level than children in the control group. Treatment with the FDA-approved drug Nusinersen was correlated with decreased circulating NF-H.

Simple Plex assays can play an important role in neuroscience biomarker discovery. Using a similar control versus experimental design, investigators can compare 36 samples in each group using the 72X1 cartridge. Mix and match with other neuroscience biomarkers using our multianalyte or multiplex cartridges. Analyze up to 4 analytes in triplicate or up to 8 analytes in duplicate, all in 90 minutes or less.

- Download the Spinal Muscular Atrophy Publication
- Purchase the Simple Plex NF-H Assay
- Explore Neuroscience Biomarkers Validated for CSF

SIMPLE PLEX™ TRACKS NEUROINFLAMMATION QUANTITATIVELY FOLLOWING TRAUMATIC BRAIN INJURY

To improve the diagnosis and prognosis of Traumatic Brain Injury (TBI), researchers today need a method to screen for TBI markers from peripheral blood. While multiplexed immunoassays can exhibit lower sensitivity and reproducibility due to cross-reactivity, Simple Plex multianalyte immunoassays avoid that challenge with separate, parallel detection of each protein target.

When neuroinflammatory biomarkers are released from neurons post-brain injury, their presence can negatively affect brain function by increasing inflammatory cytokine levels that lead to neural damage. To provide a better picture of the TBI process, Mike Anderson and colleagues at R&D Systems evaluated multiple neuroinflammatory markers using Simple Plex multi-analyte immunoassays run on the Ella platform. Simple Plex easily quantified low abundance inflammatory markers, demonstrating the broad utility of this immunoassay. Simple Plex assays provide picogram sensitivity and automated workflows, ensuring consistent and robust data across users and geographies.

- Download the TBI Poster
- Explore Simple Plex Assays for Inflammatory Markers

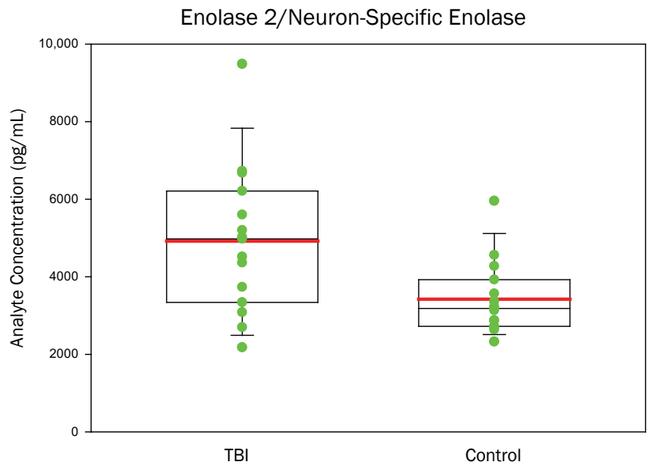

FIGURE 6. Scientists at R&D Systems evaluated whether the Simple Plex™ immunoassay could efficiently identify critical TBI blood biomarkers such as Enolase-2 (a key marker of neural injury in CSF for TBI).

CHAPTER 4: GEL AND BLOT IMAGING WITH FLUORCHEM

FLUORCHEM IMAGERS ARE THE TRUSTED SOLUTION FOR YOUR RESEARCH NEEDS.

FluorChem Imagers enable fast, high-resolution digital imaging of gels and blots. A large dynamic range allows you to get more quantitative data than film while saving you on costs. FluorChem Imagers can be found in thousands of publications and labs worldwide. With decades of experience behind our technology, our imagers are trusted everywhere for Western blot research.

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- Click Here to View the Imaging App Note Library

Click Here to View a Quick FluorChem Video

FLUORCHEM M CAN DETECT LOW LEVELS OF PARKINSON'S PROTEIN EXPRESSION.

Lewy Body formation is one of the hallmarks of Parkinson's Disease. Lewy bodies are aggregates of α-synuclein amyloid and the role they play in the disease is not clearly understood, however, Lewy bodies have been associated with both neuroprotection and toxicity. Researchers used three different methods to induce Lewy Body formation in cells, in order to gain insight into the role they play in disease progression hoping to uncover a new platform for therapeutic discovery.

As part of this study, the researchers used Western blot analysis to look at the distribution of α-synuclein and its aggregates in SH-SY5Y cells using GFP-tagged α-synuclein constructs. A ProteinSimple FluorChem M system was used to image the Western blot in order to observe the expression levels of endogenous α-synuclein and α-synuclein-GFP in these cells. With the FluorChem M imager, the researchers were able to adjust their exposure levels to detect low levels of endogenous α-synuclein expression (**FIGURE 7**).

- Click Here to Download the Paper

FIGURE 7. Expression levels of endogenous α-synuclein and α-synuclein-GFP in SH-SY5Y cells. Adapted with permission from Raiss et al. (2016) *Scientific Reports*. (CC BY 4.0).