

Biomarkers

Win the race to discovery with Simple Western™ and Simple Plex™

Speed up your biomarker discovery with Simple Western and Simple Plex from ProteinSimple. These highly sensitive, robust and hands-free platforms enable biomarker characterization in hours versus days helping you to win the race to your next discovery.

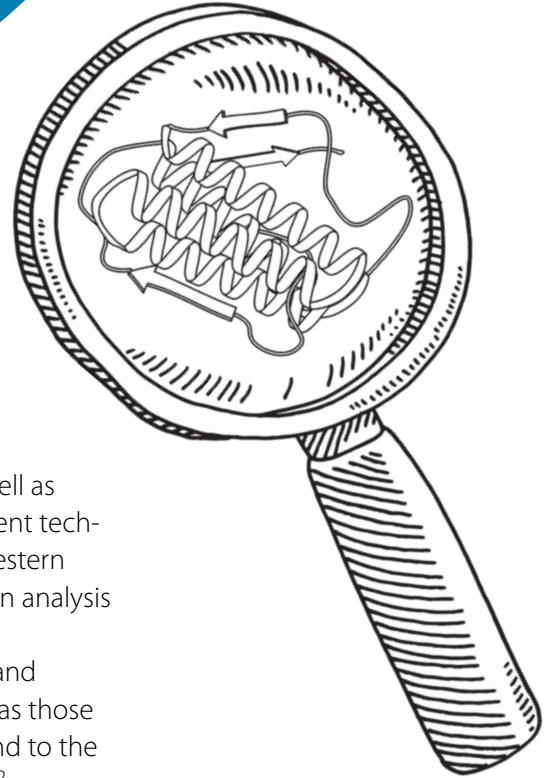
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For more information, visit
proteinsimple.com



Introduction



About Biomarkers

Biomarkers are used to screen for, diagnose, and monitor diseases as well as generate therapies and measure responses to therapeutics.¹ Many different techniques for biomarker discovery exist, but only Simple Plex and Simple Western technologies give you the sensitivity, speed, and reproducibility in protein analysis needed to win the race to biomarker discovery.

Techniques presently used for biomarker discovery include SELDI-TOF and MALDI-TOF Mass Spectrometry. However, low abundance proteins such as those released by a few tumor cells or their microenvironment don't always bind to the biochip, limiting its ability to detect low abundance molecules in serum.²

Traditional Western blots and ELISAs, two other techniques that have helped scientists discover and analyze biomarkers, also present several limitations. Traditional Westerns require multiple hands-on steps and usually take several days to obtain results. ELISAs also have multiple hands-on steps with the added problem of cross reactivity, especially with multi-analyte detection and the need to identify non-cross reactive capture and detection antibody pairs.

Simple Western and Simple Plex instruments provide higher sensitivity, faster time to results, and more reproducible data when compared to traditional Western blots and ELISAs. In this e-book, we demonstrate how Simple Western and Simple Plex have aided scientists in their biomarker discovery and analysis.

References

1. Etzioni, R. et al. The case for early detection. *Nat. Rev. Cancer* 3, 243–252 (2003).
2. Diamandis, E. P. Mass spectrometry as a diagnostic and a cancer biomarker discovery tool opportunities and potential limitations. *Mol. Cell. Proteomics* 3, 367–378 (2004).

About Simple Western

Gel-free. Blot-free. Hands-free. Simple Western.

Proteins are complex and unique molecules, which makes their analysis challenging. The most widely used protein analysis technique in existence today is the Western blot. Unchanged since its invention in 1979, it is plagued by poor reproducibility, lack of accurate quantitation, and lengthy time to result.

Simple Western changes all that by reinventing how Westerns are done. Automating all steps from protein loading and separation, immunoprobining, washing, detection, and analysis of data, it finally gives researchers an analytical tool that gives them quantifiable and reproducible data in hours versus days.

With a choice of either size (immunoassay or total protein for proteins spanning 12-440 kDa) or charge (for immunodetection of charge isoforms), Simple Westerns can analyze a broad range of proteins.

[Click here to view an introductory webinar](#)

In this webinar, we introduce you to the technology, show you how scientists around the world are using Simple Western to advance their research and how we work to help you transfer your assays with in-house antibody certification.

- [Click here to learn more about Simple Western](#)
- [Click here to learn more about Wes](#)
- [Click here to view a Wes introductory video](#)
- [Click here to view Simple Western's technical library](#)
- [Click here to learn more about Peggy Sue](#)



Detection of ovarian cancer biomarkers in cell lines with small sample sizes

Major research. Minor sample size.

Winston Kuo, President, Predicine Holdings Ltd, and his associates at Harvard Medical School used the Simple Western size assay to compare the expression patterns of three oncogenes and tumor suppressors in a normal ovarian epithelium cell line, four human ovarian cancer cell lines, and two patient samples. Due to limited tissue samples, they needed a highly sensitive detection method to analyze them. As it turns out, with Simple Western they only needed 2 µg of each patient sample to test protein expression patterns of the proteins of interest.

Not only did they successfully screen cell and tissue lysates to detect the relative levels of protein expression, they also made a substantial discovery. They found that the molecular weights of β -catenin detected in different clinical cancerous cell lysates (sample 330 and RB088) were 60 and 68 kDa respectively whereas in normal control cells (HOSE) they were at 87 kDa, suggesting there are possible genetic deletions of β -catenin in these tissue lysates. This corroborates previous data of β -catenin gene deletions linked to some populations of ovarian cancer patients and makes it a potential biomarker for the malignancy.

For these scientists, Simple Western provided a more sensitive and quantitative profile for the expression of the tumor suppressors. They concluded that the Simple Western size assay will accelerate protein detection via immune detection in the personalized and translational medicine era, providing a significant benefit to patient care and patient outcomes.

• [Click here to view this paper](#)

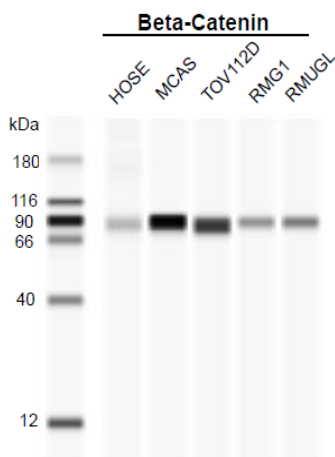


Figure 1: Cell lysates. Normal and ovarian cancer cell lysates analyzed by Simple Western size analysis.

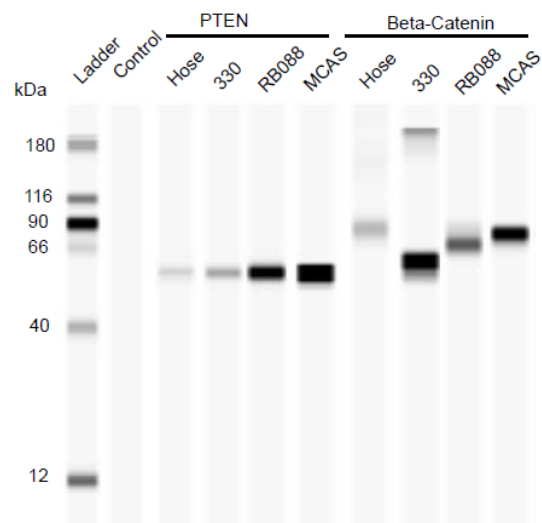
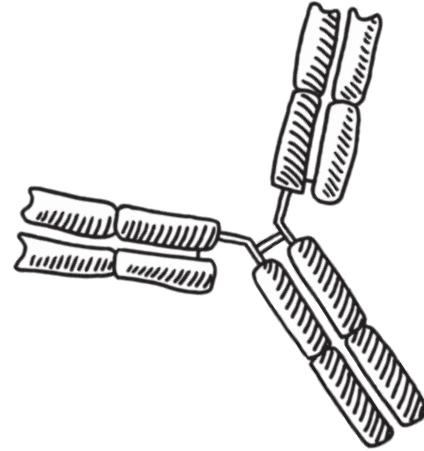


Figure 2: Protein Expression. Expression profiles of Beta Catenin and PTEN in ovarian patient tissue. Normal and ovarian cancer cell lysates were used as positive controls.

Wes gives insight into complex formation of serotonin receptors in the brain in a fraction of the time



Wes' fast time to results allowed these scientists to do some speedy biomarker discovery.

Addiction is a brain disease in which changes in neural structure and function create a barrier to abstinence. In this webinar, Dr. Noelle Anastasio with graduate student Sarah Swinford-Jackson have used Wes to understand the relationship between serotonin receptors and impulsivity in laboratory mice.

After discovering that levels of serotonin 2a and 2c receptors in the prefrontal cortex are associated with impulsivity, they hypothesized that receptors 2a and 2c could form a protein complex. To prove this hypothesis, they used co-immunoprecipitation to look at receptor interactions in

the pulldown fractions on Wes. They found that receptors 2a and 2c form a protein:protein interaction which is lower in high impulsive versus low impulsive rats suggesting that the complex may act as a regulatory control over the prefrontal cortex. And they were able to do this much faster with Wes since it only took them 4 hours to generate data compared to the 4 days it would take with traditional Western blotting.

- [Click here to view this webinar](#)
- [Click here to view this From Your Peers article](#)

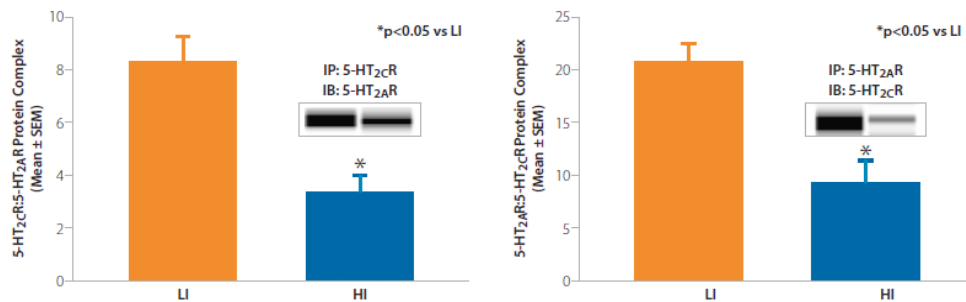
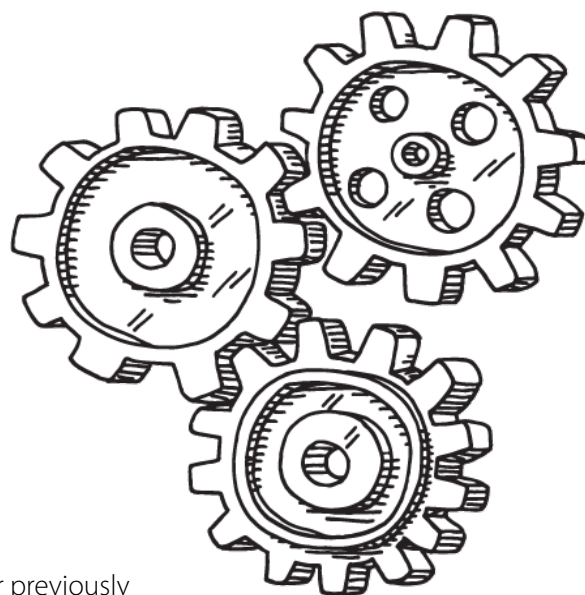


Figure 3: Protein:Protein interactions in the prefrontal cortex. Reciprocal pulldown assays of protein immunoprecipitated from medial prefrontal cortex of low impulsive (LI) and high impulsive (HI) mice to understand protein:protein interactions of serotonin 2a (5-HT_{2a}R) and 2c (5-HT_{2c}R) receptors. Reprinted with permission from Anastasio NC, Stutz SJ, Fink LHL, Swinford-Jackson SE, Sears RM, DiLeone RJ, Rice KC, Moeller FG and Cunningham KA (2015) ACS Chemical Neuroscience 6 (7): 1248-1258. Copyright 2015 American Chemical Society.

Simple Western charge assays help create a robust test for ASNS expression



Charge your way to high throughput.

Dr. Phil Lorenzi and his team at the MD Anderson Cancer Center previously found that asparagine synthetase (ASNS) has a negative correlation with L-ASP anticancer activity in ovarian cancer cell lines, which suggests that L-ASP is an effective treatment for ovarian cancers with low-ASNS. In this webinar, Dr. Lorenzi explains how he used NanoPro 1000 to develop a quantitatively robust single-antibody Simple Western charge assay for ASNS expression.

Assay robustness was key to them and they were delighted when Simple Western exceeded their expectations. They discovered a single ASNS isoform and, under optimized conditions, the assay yielded less than 8% CV, a 60-fold dynamic range, and Z'factor of 0.82, indicating a robust, high throughput assay. This proves that Simple Western gives them a robust high-throughput charge assay with preliminary results also showing more resiliency to the matrix effect than ELISA.

- [Click here to view this poster](#)
- [Click here to view this webinar](#)
- [Click here to learn more about NanoPro 1000](#)

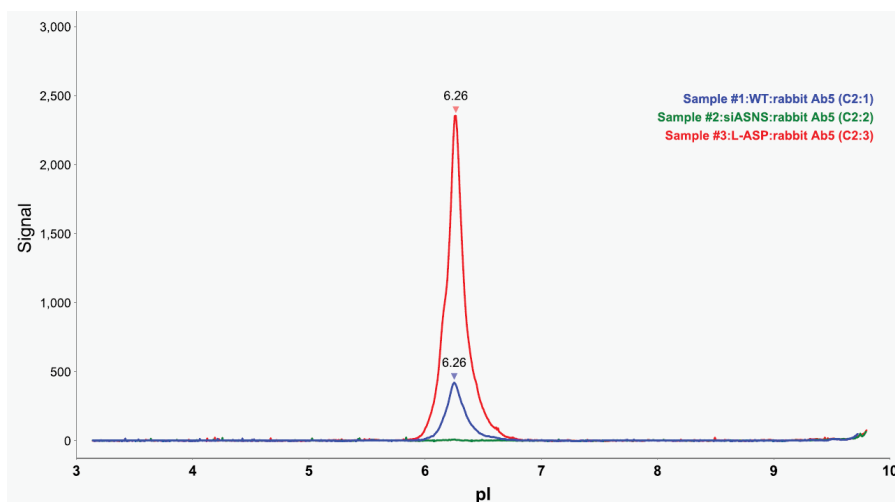
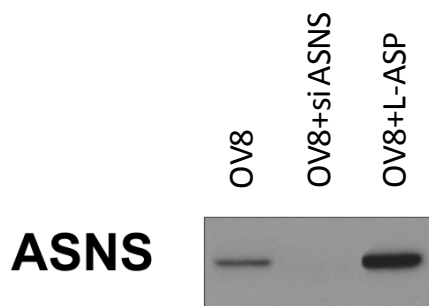


Figure 4: Characterization of NanoPro signal specificity. The levels of ASNS increase in L-asp treated samples and decrease in siRNA samples, which correlates with traditional Western blot data.

About Simple Plex

Ella makes biomarker discovery a breeze.

Simple Plex is the leading developer of high performance immunoassay solutions for single analyte and multi-analyte formats. Here we show why Simple Plex is the very best for low abundance cytokines and other bioactive proteins. While standard ELISA assays often have low sensitivity due to analyte cross-reactivity and complex assay set-up, Simple Plex eliminates these challenges entirely. Its novel automated cartridge simultaneously measures 1-4 analytes in a low volume format that is run on the Ella analyzer, and is backed by R&D Systems reagents for the highest quality. Simple Plex is ideal for quantitating protein biomarkers like cytokines in biological samples such as human plasma/serum and CSF. It also offers significant benefits for clinical research applications. In this section of the e-book, we'll introduce you to the latest information from Simple Plex end-users and our internal R&D scientists.

- [Click here to learn more about Simple Plex](#)
- [Click here to learn more about Ella](#)
- [Click here to view Ella's video](#)
- [Click here to view the Simple Plex technical library](#)



Get rapid biomarker analysis with Simple Plex

Ella's higher sensitivity and dynamic range performs better than plate-based ELISAs.

In this poster, Preeti Kapoor of ProteinSimple shows the efficiency of using the Simple Plex platform for biomarker screening in blood serum and other sample types using both the multi-analyte cartridge (16 samples for 4 analytes) as well as the new single analyte cartridge (72 samples for 1 analyte) on the Ella instrument. The workflow demonstrates the speed and ease of the validated assay setup, with no manual washes and the use of factory-generated standard curves. The data confirm that Simple Plex's 72x1 cartridge accurately detects down to picogram/mL limits consistently for IL-6 cytokine and other analytes, with more sensitive results at a 25 μ L sample volume than from a high quality plate-based ELISA. In addition to higher sensitivity, Simple

Plex also offers a greater dynamic range at 4-5 logs than plate-based ELISAs, which enables detection of endogenous levels in normal samples and disease states simultaneously.

- [Click here to view this poster](#)
- [Click here to view Ella's brochure](#)

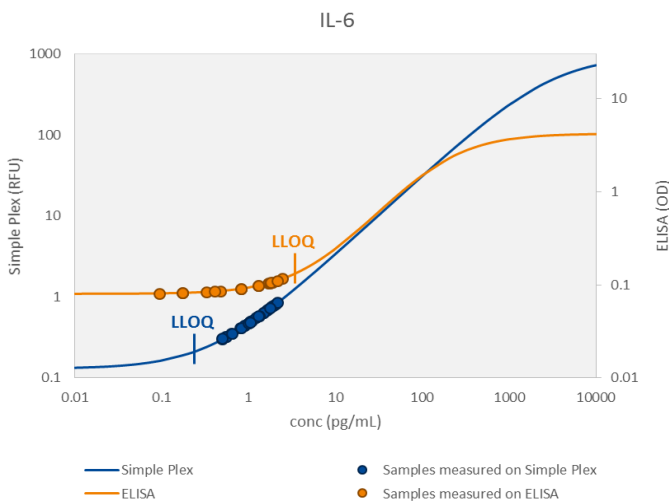


Figure 5: Simple Plex's single analyte cartridge (72x1) versus ELISA. Simple Plex demonstrates more sensitive results versus a high quality plate-based ELISA.

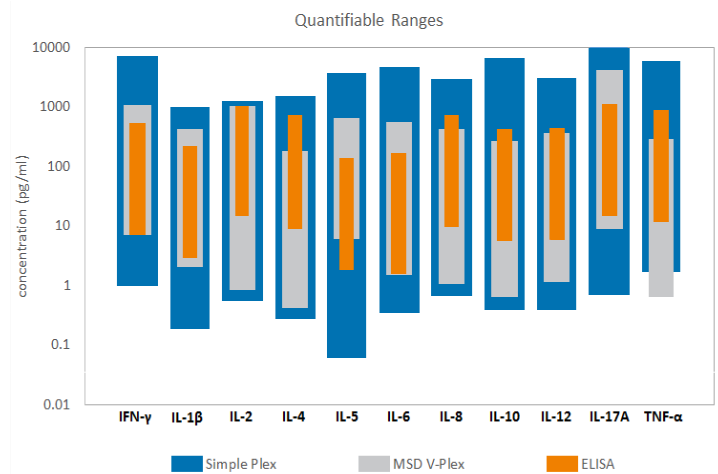


Figure 6: Dynamic range of Simple Plex versus ELISA. Simple Plex offers greater dynamic range at 4-5 logs for a broad range of cytokines compared to plate-based ELISA and other technologies.

Ella illuminates the effect of biomarkers on Traumatic Brain Injury

Not a lot of sample? Ella doesn't mind.

There are many proteins in disease states which are hard to consistently detect in the blood, because they are at such a low concentration. For a series of chemokines linked to Traumatic Brain Injury, Simple Plex makes that task easy.

Post-brain injury proinflammatory biomarkers are released to induce a neuroinflammatory response. However, the prolonged presence of these biomarkers negatively affects neurons and brain function by causing increased cytokine production and neural damage. Mike Anderson and his colleagues presented a poster at Neuroscience 2015 in which they evaluated multiple neuroinflammatory biomarkers using Simple Plex which allows for a more accurate diagno-

sis of Traumatic Brain Injury.

Simple Plex's multi-analyte cartridge format can easily confirm quantitative levels, because the assay is that robust at low-level detection. So even if you have several researchers using the assay, or your sample pipetting varies, the assay is very tolerant and will still provide you with excellent and reproducible results, at picogram/microliter levels.

- [Click here to view this poster](#)
- [Click here to view a brochure or datasheet](#)

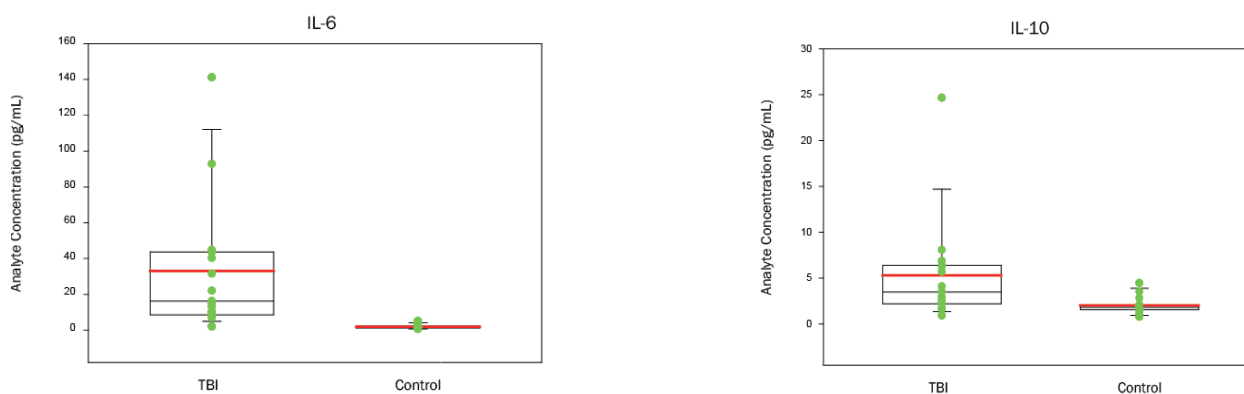


Figure 7: Proteins that could act as markers for Traumatic Brain Injury. IL-6 and 10 are just two of many analytes with increased concentrations in Traumatic Brain Injury samples compared to control samples.

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Dr. Phil Lorenzi, MD Anderson Cancer Center

Preeti Kapoor, ProteinSimple

Mike Anderson, R&D Systems

The Simple Western Family



NanoPro 1000

Charge assays on up to
96 samples

[Request Pricing](#)



Wes

Size assays on up to
25 samples

[Request Pricing](#)



Peggy Sue

Size and charge assays
on up to 96 samples

[Request Pricing](#)

Sally Sue

Size assays on up to
96 samples

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Simple Plex



Ella

Single or Multi-Analyte
immunoassays

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