proteinsimple™

The Simple Western™: a gel-free, blot-free, hands-free Western blotting reinvention

Western blotting is considered the gold standard for protein detection and characterization. Although improvements to individual aspects of Western methodologies have been developed in recent years, none has integrated the entire process onto a single platform. ProteinSimple™ has developed Simple Western™ assays for protein sizing and quantitative immunodetection as an alternative to traditional Western blot analysis. Assays are performed on Simon™, an instrument that integrates and automates all manual operations associated with Western blotting.

Introduction

Western blotting is the most widely used and accepted methodology for protein detection and was first reported in the literature over 30 years ago[1,2]. Although Western blotting is a proven technique, it is plagued by poor reproducibility, lack of accurate quantitation, extensive time to result and reliability issues. Improvements have been made to reagents and individual steps within the Western blotting process over the years, but none has fully overcome the challenges and bottlenecks still experienced by researchers today. The Simple Western is a reinvention of the entire Western blot, automating all steps from protein loading and separation, immunoprobing, washing, detection and quantitative analysis of data, finally giving researchers a complete, walk-away solution. Manual factors that can negatively impact reproducibility, quantitation, time to result and overall reliability of the generated data are eliminated.

Simple Western basics

Samples are prepared following conventional procedures[3]. Samples are then mixed with Simple Western Sample Buffer and standards to a final concentration of 1 µg/µL, reduced and denatured. The prepared samples, primary and secondary antibodies and chemiluminescent substrate are dispensed in microliter volumes into designated wells in a low-volume 384-well assay plate. Simple Western assay buffers, nano-volume capillaries and the prepared assay plate are placed in Simon, which carries out all assay steps automatically. Proteins are separated in capillaries as they migrate through a stacking and separation matrix. The separated proteins are immobilized to the capillary wall via a proprietary, photoactivated capture chemistry. Target proteins are then identified with a primary antibody and subsequent immunodetection using a horseradish peroxidase (HRP)-conjugated secondary antibody and chemiluminescent substrate. Molecular weight and signal for immunodetected proteins are automatically reported. Simultaneous analysis of up to 12 samples can be performed in a single experiment, and results are available in 3–5 hours. The software reports molecular weight, area, percent area and signal to noise for each protein detected.

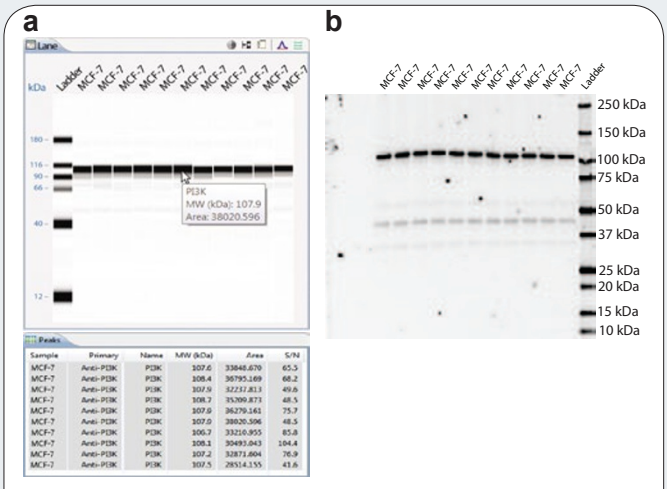

Figure 1 | Comparison of phosphoinositide 3-kinase detection via Simple Western and Western blot. Ten replicates ($n = 10$) of MCF-7 lysate (Santa Cruz Biotechnology p/n SC-2206) at 1 µg/µL were analyzed using a PI3K-specific primary antibody (Cell Signaling p/n 3011). The predicted molecular weight of PI3K is 110 kDa. (**a**) Simple Western lane view with results automatically analyzed by system software. (**b**) Western blot image captured on FluorChem M with results analyzed manually in AlphaView software (both ProteinSimple). Replicate data averages are summarized in **Table 1**.

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APPLICATION NOTES

Table 1 | Summarized results for the Simple Western and Western blot data shown in Figure 1.

PI3K	MW (kDa)	% CV (MW)	Signal	% CV (Signal)	Signal to noise
Simple Western	107	0.5	33747	8.7	66
Western blot	114	2.2	212295	8.7	9.3

PI3K, phosphoinositide 3-kinase. MW, molecular weight. CV, coefficient of variation.

More quantitative and reproducible results

Reproducibility of results from a traditional Western blot is a common challenge for researchers due to lack of standardized procedures and the multiple handling steps that introduce experimental variability. Because the Simple Western assay is fully automated, results are more reproducible than those generated via Western blot. Overall quantitation is vastly improved as blot transfer is not required, thus eliminating any inconsistencies in protein transfer. **Figure 1** demonstrates the reproducibility and accuracy of a Simple Western assay compared to Western blot for detection of phosphoinositide 3-kinase (PI3K) expression in an MCF-7 lysate. Simple Western assay data (**Fig. 1a**) is represented by a software-generated lane view image, and protein size, signal intensity and area of the chemiluminescent signal are reported. Western blot data (**Fig. 1b**) was generated following a standard protocol, and the fluorescent image was captured using a traditional imager and analysis software. Results are summarized in **Table 1**.

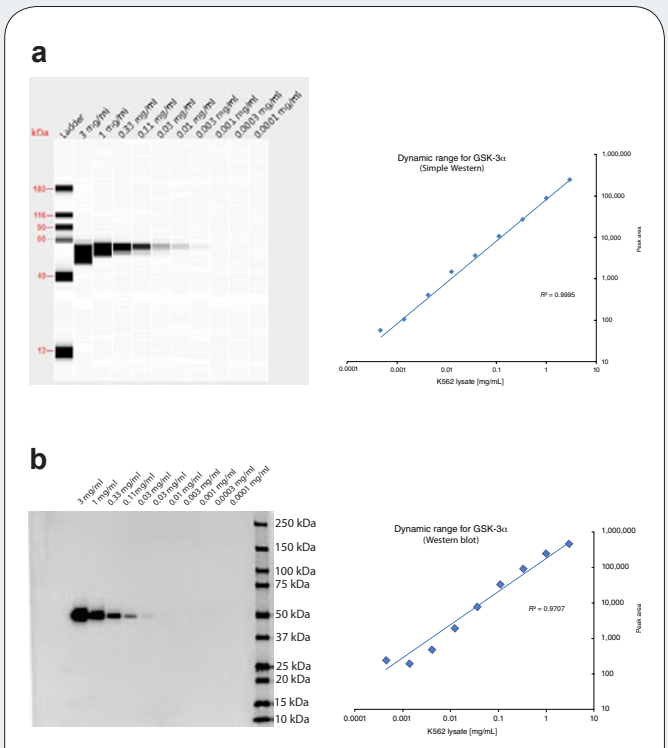

Figure 2 | Comparison of Simple Western and Western blot dynamic range. K562 cells lysed in Bicine/CHAPs buffer were serially diluted and analyzed using a glycogen synthase kinase-3α (GSK-3α) antibody (Cell Signaling p/n 4818). (**a**) Simple Western lane view with quantitative results automatically generated in system software. (**b**) Western blot results captured using FluorChem M with quantitation manually performed using AlphaView software (both ProteinSimple). Coefficient plots were generated in Microsoft® Excel® for both methods.

Wider dynamic range

Simple Western assays have a linear dynamic range of approximately three orders of magnitude depending upon the protein target. As shown in **Figure 2a**, the dynamic range for glycogen synthase kinase-3α (GSK-3α) in a K562 lysate was 3.3 logs with an R^2 value of 0.999. For Western blot analysis on the same lysate samples using the same antibody (**Fig. 2b**), a less linear response was observed, with a dynamic range of 2.5 logs and an R^2 value of 0.971.

Summary

The Simple Western is the first fully automated and complete solution for protein detection and characterization, representing a true reinvention of Western blotting. Researchers are now able to simply load their samples, press start, walk away and return a few hours later to fully analyzed experimental results. Simon automates the entire process from start to finish and eliminates all hands-on labor, offering significant time savings and drastically decreasing time to result. The high quality of data generated is considerably more reproducible between users and over time. In addition, the process variability, blot transfer and manual analysis that made traditional Western blot results semi-quantitative at best are eliminated, allowing highly quantitative results to be obtained over a wide dynamic range. Up to 12 samples can be analyzed in 3–5 hours, and targets between 15–150 kDa can be detected. Simple Western assays run on Simon also facilitate standardization of laboratory processes, and provide data in a format that can be easily shared between multiple users and facilities. For more information please visit proteinsimple.com

1. Towbin, H. *et al*. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354 (1979).
2. Renart, J. *et al*. Transfer of proteins from gels to diazobenzyloxymethyl-paper and detection with antisera: a method for studying antibody specificity and antigen structure. *Proc. Natl. Acad. Sci. USA* **76**, 3116–3120 (1979).
3. Gallager, S.R. One-dimensional SDS gel electrophoresis of proteins, basic protocol 1. In *Current Protocols in Protein Science* (eds. Coligan, J.E. *et al*.) **10.1**.1 -10.1.34 (John Wiley & Sons, Somerset, N.J., 1995).