Accurate Quantitation of Recombinant Proteins with Simple Western

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Abstract

Why do researchers use Western blots? It's been the default technology for confirming the presence or absence of a protein, but Western blots are a poor method for measuring the amount of that protein. Accurate quantitation of proteins using traditional Western blotting has been a goal since the technique was developed over thirty years ago. However, because the process requires many steps, each introducing variability, it has not been possible. Portions of the technique have been automated to try and improve consistency, but until the Simple Western, there had been no major leap in any technology that would propel this method of protein analysis from qualitative to quantitative.

The Simple Western platform is the modern evolution of the traditional Western blot. The fully automated system is easy to use and removes variability so results are more reproducible run to run, between users and over time. Since a blotting step is not performed, protein transfer inconsistencies are eliminated, providing more consistent results. Utilizing the curve-fit feature in the software provided with the system allows comparison of recombinant proteins in a sample against a standard curve. These features of Simple Western allow researchers to not only identify their protein, but also achieve reliable quantitation of recombinant proteins.

We present data comparing quantitative analysis of AKT1 via traditional Western blot and Simple Western on Wes, highlighting assay workflow, biological response, sensitivity, and resolution.

Assay Principles

Wes is a bench top instrument capable of running up to 25 samples simultaneously to separate proteins by size (Figure 1). The assay kits come with a pre-filled plate that contains all the reagents other than the researcher's samples and primary antibodies (Figure 2). Samples for Wes are separated in a self-contained capillary cartridge, and immobilized to the capillary wall via a proprietary UV capture method. Target proteins are immunoprobed with an antibody followed by HRP-amplified chemiluminescent detection (Figure 3). Wes automates the entire Western blot procedure increasing reproducibility and delivering significant workflow time savings.

FIGURE 1. Wes

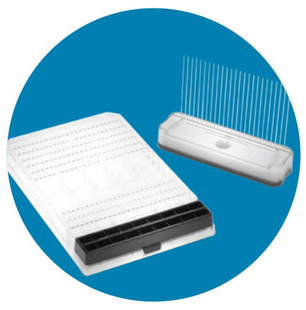

FIGURE 2. Capillary cartridge and pre-filled plate

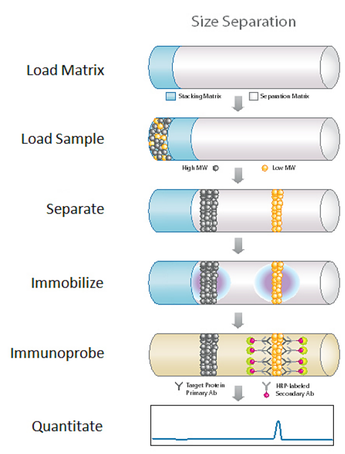

FIGURE 3. Steps of the Wes Assay

Workflow Comparison

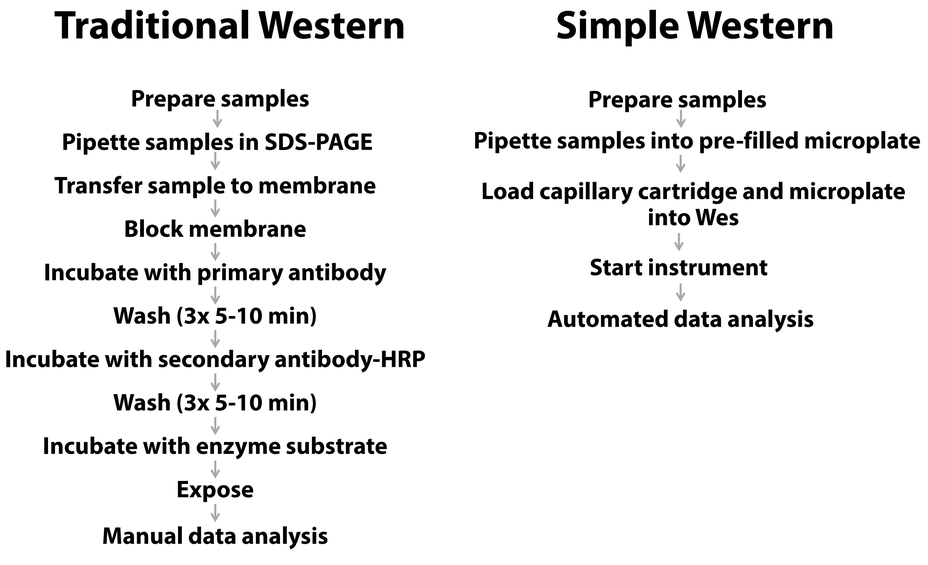

FIGURE 4. Workflow comparison for Simple Western and Traditional Western

Comparison of Quantitation Accuracy

Comparing the accuracy of quantitation of endogenous AKT1 in Jurkat lysate between Wes and a traditional Western blot. GST-labeled AKT1 was used to generate a standard curve for quantitation of endogenous AKT1.

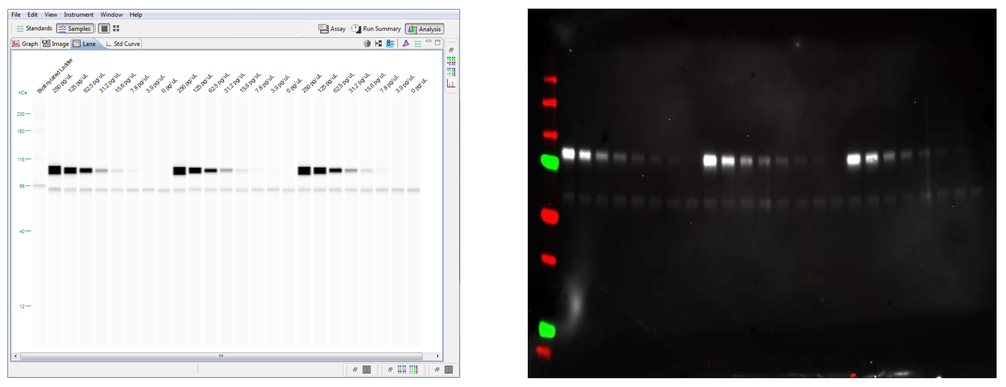

FIGURE 5. AKT1-GST was spiked into the Jurkat lysate at decreasing concentrations (250-0 pg/µL). Both labeled and endogenous proteins were detected using an AKT1 monoclonal antibody. Equivalent concentrations of protein were loaded in each well of the Wes assay plate and each well of a 10% acrylamide SDS-PAGE gel. The plate and capillary cartridge were inserted into Wes and the assay run was started. For the traditional Western, the gel was run at 100V until the dye front reached the bottom of the gel. Transfer was done at 100V for 1 hr 15 minutes. The membrane was then blocked for 1 hr and incubated with the primary antibody overnight at 4° C. This was followed by 1hr incubation with goat anti-rabbit-HRP secondary antibody. The bands were detected with Dura luminol/peroxide and the imaged on the FluorChem M using an 18 minute exposure.

FIGURE 6. Analysis of peak area in an electropherogram for Simple Western and image analysis of the traditional Western. The Compass software for Simple Western automatically calculates the peak area of the fitted peaks for GST-AKT1 and AKT1. The imager software requires manual selection of the bands in the image of the traditional Western blot. The width of the detection area is selected by the software and cannot be altered by the user.

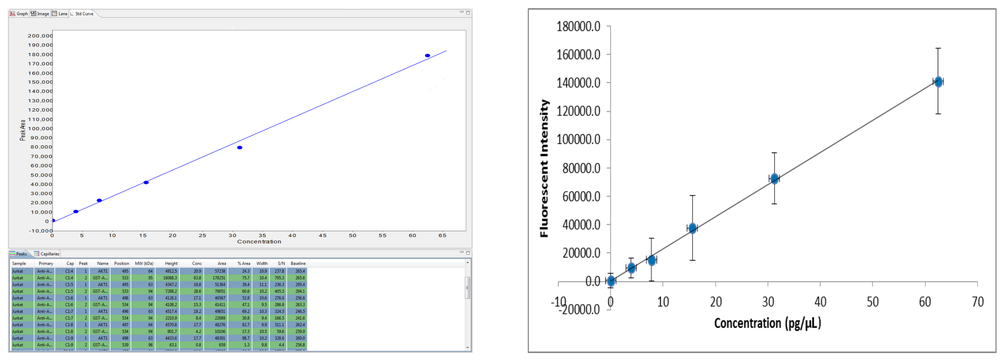

FIGURE 7. Linear regression analysis and quantitation of endogenous AKT1. Wes automatically generates a linear curve fit and calculates the endogenous AKT1 with the Compass software. To generate a linear curve fit for a traditional Western, it is necessary to export the data from the image analysis software to another software program, average the signal strength for each point of the standard curve, and calculate the concentration based on the standard curve. Because the signal for Wes and the traditional Western had reached saturation at 62.5 pg/µL, the top two standards were removed to improve the curve fit.

Assay Comparison

	Wes (Simple Western)	Traditional Western
Hands-on time	30 minutes	120 minutes
Time to results	3 hours	2 days
Calculated concentration of endogenous AKT (Average of n = 3)	21 pg/µL	12.5 pg/µL
Concentration range	16.1 – 21.3 pg/µL	6.2 - 20.2 pg/µL
Quantitation CV	9.4%	34.5%

FIGURE 5. Comparison of Wes and traditional Western

Conclusion

- Wes delivered truly quantitative size-based separation of AKT1.
- Wes generated a highly reproducible and sensitive assessment of endogenous AKT1.
  - The CV for detection of endogenous AKT1 in Jurkat lysate was 9.4% at a concentration of 21 pg/µL.
- Wes streamlines the total time to results to less than 3 hours in a walk-away experiment that requires only 30 minutes of hands-on time.