Comparison of Conventional Western Blot Analysis with a Fully Automated Capillary Electrophoresis System for Size-based Analysis of the AKT Pathway Signaling Cascade

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Abstract

Protein kinase AKT is a central player for a variety of cell stimuli affecting downstream events such as the inhibition of apoptosis, regulation of glycogen synthesis, and cell cycle regulation. Many of these events are routinely assessed by Western blot analysis which exploits high sensitivity of enzyme amplification as well as specificity of antibody binding. The process itself, however, is very labor intensive and has not significantly changed since it was first introduced.

Capillary electrophoresis is a powerful separation technique with high resolution and reproducibility. In proteomic analysis, Western blots are limited to validation of selected targets due to lack of automation, while capillary electrophoresis is restricted to purified or enriched protein samples with very low throughput. ProteinSimple's technology incorporates sample separation performed in capillaries followed by UV-triggered immobilization of the proteins directly onto the capillary walls, followed by immunodetection as demonstrated already by our NanoPro 100 and NanoPro 1000 systems for IEF (isoelectric focusing). Simon, a new system from ProteinSimple, runs fully automated Simple Western assays.

We present data comparing analysis of key targets of the AKT signaling cascade via Western blot and Simple Western on Simon, highlighting workflow, biological response, sensitivity, and resolution.

Conclusion

- Simple Western assays run on Simon are a reinvention of the Western blot, automating all the manual steps associated with the process.
- Simple Westerns generate highly reproducible, quantitative, reliable and sensitive assessment of key targets in the AKT signaling cascade.
- Simple Westerns streamline the total time to results to about 3-5 hours in a walk-away mode with a total hands-on time of less than an hour.

Assay Principles

Simon is a bench top instrument capable of running 12 samples simultaneously with Simple Western assays, which are size-based assays equivalent to SDS-PAGE (Figure 1). Samples for Simple Western assays are treated with SDS/DTT and heat denatured. Samples are then loaded into capillaries, separated by size 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 2). Simon allows for the automation of the entire Western blot procedure, which results in increased reproducibility and significant time savings.

FIGURE 1. Simon

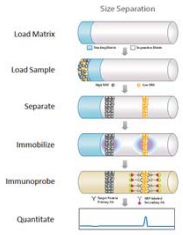

FIGURE 2. Steps of a Simple Western Assay

Workflow Comparison

Traditional Western	Simple Western
Load samples in PAGE	Load plate in Simon
Transfer sample to membrane	Start instrument
Block membrane	Integrated data analysis
Incubate with primary antibody	
Wash (3x 5-10 min)	
Incubate with secondary antibody-HRP	
Wash (3x 5-10 min)	
Incubate with enzyme substrate	
Expose	
Manual data analysis	

Figure 3: Workflow comparison for Simple Western and Traditional Western

Simple Western Assay Specifications

Description	Specifications
Sample required	~3 µg
Run time	3-5 hrs
Sizing range	15–150 kDa
Sizing accuracy	+/– 20%
Sizing CV	10%
Resolution	+/– 10% difference in molecular weight
Quantitation CV	≤ 20%
Dynamic Range	3 logs
Sensitivity	Low ng
Samples per run	12

Figure 4: Simple Western Assay Specifications

Comparison of Dynamic Range

Figure 5 compares the dynamic range of the Simple Western to the traditional Western blot. Simon provides a dynamic range over three orders of magnitude for GSK3α in K562 cells, while providing a more linear fit of the data over this range.

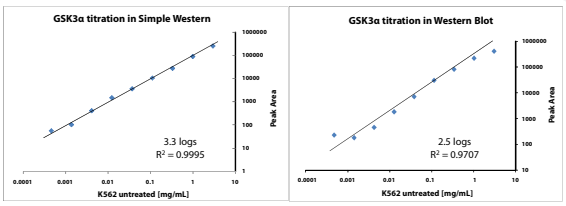

Figure 5: K562 cells were lysed in Bicine/CHAPS Lysis Buffer (ProteinSimple) and run via Simple Western and Western blot. Both were probed with α-GSK3α (4818) antibody from Cell Signaling.

Detection Using Multiple Antibodies in a Single Run

By using independent capillaries for each data point, Simon facilitates the use of many different antibodies in one run as compared to a traditional Western. Figure 6 illustrates the detection of GSK3β expression via Simple Western using multiple antibodies without the need to perform separate experiments for each antibody. The equivalent Western blot data shown required the handling and processing of four different blots.

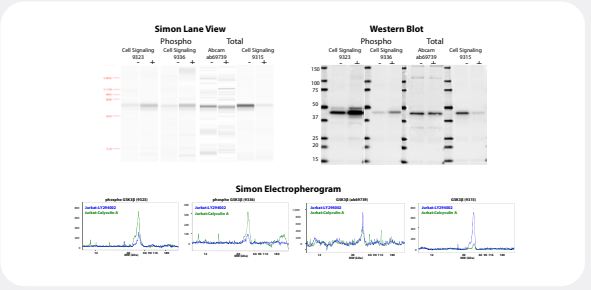

Figure 6: Lysates from Jurkat cells treated with 50 µM LY294002 or Calyculin A from Cell Signaling (9273) were run via Simple Western and Western blot and probed with various GSK3β antibodies.

Detection of AKT Pathway Key Targets

Equivalent biological activity of several targets in the AKT pathway is observed using Simple Western assays and Western blot. Simon's integrated software analysis features allow for review of the separation, simple quantitation and presentation of results in several different views.

Figure 7: Simple Western and Western blot results for six different members of the AKT pathway. Commercial lysates as indicated in individual legends were run using 3 µL of a 1 mg/mL final concentration for each method.