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WHITE PAPER

SIMPLE WESTERN PRODUCES PUBLICATION-READY RESULTS

PRODUCING PUBLICATION-QUALITY DATA WITH WESTERN BLOT IS CHALLENGING AND PRONE TO MISREPRESENTATION. SIMPLE WESTERN CHANGES THAT.

MEETING NEW SCIENTIFIC STANDARDS

Specific detection of proteins is central to biological research. The standard assay for accomplishing this has traditionally been the Western blot because it incorporates molecular weight information and antibody-based detection to provide high-specificity measurements of protein identity and abundance. Developed in the 1970s[1], the Western blot is traditionally a manual workflow that suffers from lack of reproducibility among other challenges. Primary among these challenges is how to analyze and publish Western blot data, which at its core is simply an image of a membrane with chemiluminescent or fluorescent signals while meeting established guidelines for reproducibility and rigor. With modern image analysis software, it is easy and therefore tempting to manipulate Western blot data and images, which can lead to a misrepresentation of the results. An eye-opening study found that approximately 25% of accepted papers contain at least one instance of an improperly modified image, and many of these images were Western blot data.[2] In addition, despite being a qualitative assay by nature, researchers have bent over backwards to glean quantitative information from traditional Western blotting images that arguably isn't there to begin with.[3,4]

To meet this challenge, the National Institutes of Health (NIH) Initiative on Rigor and Reproducibility was established to ensure robust, unbiased experimental interpretation and reporting of results and affirm the importance of data reproducibility.[5] This initiative has driven many granting agencies and journals to establish guidelines for grant writers and publication authors to improve reproducibility, transparency and rigor in grant submissions and journal articles. For example, the Guidelines for Transparency and Openness Promotion (TOP) Guidelines from the Center for Open Science have been adopted by numerous top journals including *Science*, *Cell* and *Elsevier* and establish guidelines for making raw data available in a relevant data repository to improve data transparency.[6]

Simple Western™ systems, which launched in 2011, have emerged as a powerful tool to replace traditional Western blotting workflows while meeting established guidelines on reproducibility and rigor.[7] In addition to the sensitivity and throughput advantages afforded by complete automation, Simple Western systems create electronic data files that are impervious to manipulation and contain both raw data and analysis that can be easily uploaded to a data repository when it comes time to publish.

As granting agencies and journal editors have increasingly put in place measures to ensure integrity of Western blotting datasets, publications using Simple Western systems to generate Western blotting data have grown rapidly, increasing from a half dozen in 2011 to more than 1,000 today. As such, Simple Western is poised to become the gold standard technology, replacing traditional Western blotting workflows entirely.

This White Paper examines how Simple Western overcomes the challenges and pitfalls associated with traditional Western blots to produce publication-ready data that meets the highest standards of data integrity and rigor.

THE PROBLEM WITH TRADITIONAL WESTERN BLOT DATA

Western blot data are met with three commonly encountered problems. First, images of blots are easily susceptible to misrepresentation, either by deliberate fraud or more innocent attempts to make the images 'cleaner' for publication. Examples include cutting out lanes to rearrange sample order, changing detection settings when imaging membranes or nonuniformly changing contrast settings to enhance visualization of weak bands. Unfortunately, misrepresentation of Western blot data is surprisingly common.[2] In response, journals like *Nature*, *Cell*, and *The Journal of Biological Chemistry* are specifying stricter guidelines for acceptable practice of publishing Western blot data,[8-10] and even perform spot checks from randomly chosen papers.[11]

Second, Western blot data are primarily qualitative, and extracting quantitative information is difficult and often unreliable. To translate a band on a membrane to a numerical value that can be used for statistical analysis, a researcher must rely on densitometry, an inexact practice that introduces a high amount of variability. In one study, different commonly-used densitometry procedures applied to an identical Western blot revealed *p*-values of correlations ranging from 0.000013 to 0.76.[12] Also, for accurate quantification to occur, the signal must fall within the linear range of detection, which may be unknown and is often relatively narrow. As a result, traditional Western blotting data are only semi-quantitative at best.

A third challenge with traditional Western blot data is that they are not easily sharable for data transparency. While you can upload a membrane image to a repository, the image would not contain essential information like the experimental conditions used, sample identification, antibody concentration, etc. Furthermore, the image would not contain any of the analysis like peak area and quantitation. These factors preclude the sharing of Western blot data in a data repository in a standardized and easily accessible manner.

SIMPLE WESTERN DATA ARE PUBLICATION-READY AND RICH IN QUANTITATIVE DETAIL

Simple Western technology overcomes the commonly encountered problems with traditional Western blot data by creating highly reproducible, quantitative protein data in one electronic data file which integrates experimental assay conditions, raw data and quantitative peak areas and other analyses. As a result, Simple Western data are difficult if not impossible to misrepresent if published directly from the Compass for Simple Western Software. Unlike traditional Western blots, Simple Western is highly quantitative by nature, bypassing densitometry analysis altogether. Data generated by Simple Western instruments automatically appear in the Compass for Simple Western Software once the run has completed. Here, data may be analyzed and exported directly for publication. This one-stop solution eliminates the need to pass between different pieces of equipment to analyze data, for instance the gel doc and computer and is amendable for uploading to appropriate data repositories or journals to improve transparency. If you're working in Quality Control (QC) and Good Manufacturing Practice (GMP) environments and need to manage data integrity, Compass for Simple Western has integrated functionality for compliance with 21 CFR Part 11.

Simple Western data can be viewed in a variety of ways but is always reproducible and not subject to incorrect manipulations. Simple Western users may choose to view data in graph view, lane view, and they may even view the direct image of the capillary. Among these different ways to view data, we recommend the graph view because it provides the most resolution and quantitative detail. In graph view, data is portrayed as an electropherogram, with size or charge-based migration distance on the X-axis and the signal intensity of the chemiluminescence or fluorescence readout on the Y-axis (**FIGURE 1A**). This Cartesian representation of immunoassay data provides immediate quantitative information for signal intensity, which is derived from the area under the curve. For each peak in the electropherogram,

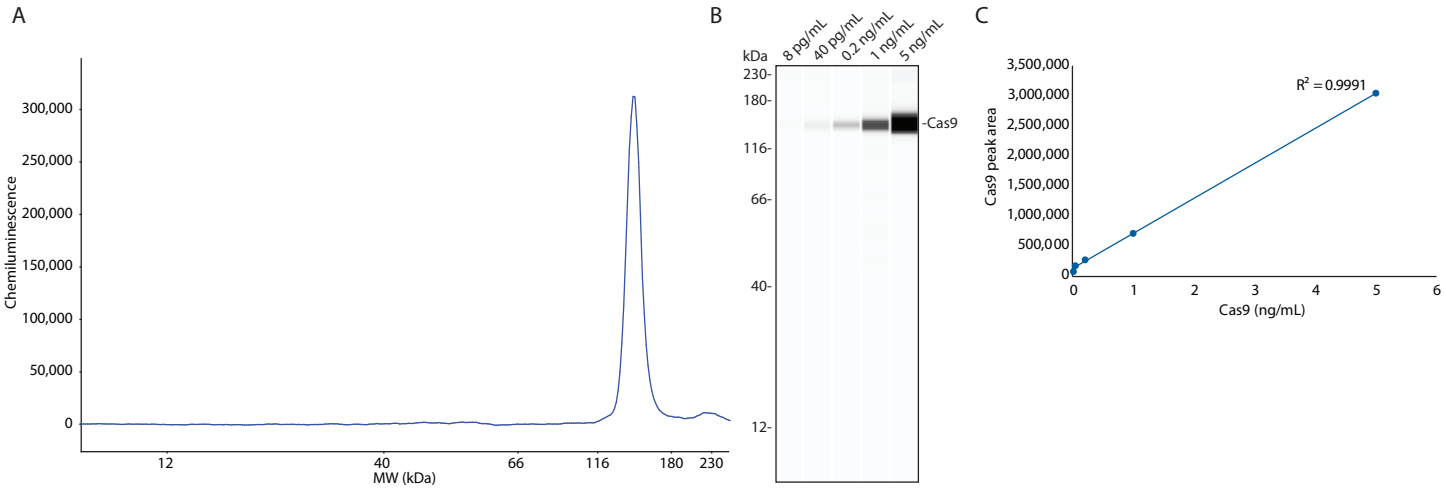

FIGURE 1. Detection of recombinant Cas9 by Wes, a Simple Western instrument. (A) Electropherogram of Cas9 detected by a chemiluminescence readout on Wes (B) lane view of Cas9 detection and (C) linearity analysis of Cas9 detection. Cas9 was detected with the Mouse CRISPR-Cas9 Monoclonal Antibody (MAB10252) from R&D Systems.

Simple Western automatically calculates signal height, area, % area, width, S/N, and baseline values, and these numbers may easily be exported for further statistical analysis if desired. Immunoassay data portrayed in this manner is not prone to the same pitfalls associated with publishing traditional Western blot data. Unlike Western blot images, the electropherogram has no contrast or exposure that can be manipulated to enhance or suppress signal. The 'peaks and valleys' of an electropherogram may better illuminate charge or size isoforms, cross-reactivity, or degradation products, as opposed to a crude image data of the traditional Western blot.

Users of traditional Western blot may not be accustomed to viewing data portrayed as electropherograms. For this reason, Simple Western also offers a lane view feature that allows for data portrayal that more closely resembles the image of traditional Western blot (FIGURE 1B). In this view, protein signal is not portrayed as in the Cartesian graph, but rather as virtually mimicked lanes on a gel. Even in lane view, Compass for Western eliminates the possibility of manipulating Western blot data that goes against data integrity guidelines. For example, the acceptable practice is that contrast and brightness of a gel image may be adjusted so long as it is applied to the entire blot.[8] In accordance with this guideline, Compass for Simple Western allows users to adjust these parameters across all samples. Because each of the capillaries are independent, lanes can also be rearranged in the software, eliminating the need to rerun samples so that they are adjacent or manually splice lanes together, which is discouraged by journals.[7,8]

For whichever view you choose, the data may be exported complete with annotations and in a high-quality format. In other words, researchers do not need to rely on 3rd party software to annotate or prepare their data for publication. The data exported from Compass for Simple Western is standardized and ready for publication without manipulation that can introduce bias or intentional misconduct. Nevertheless, it also has export functionality so that both the images and quantitative analysis can be analyzed with custom tools. For example, quantitative information can be exported as a CSV file for analysis in Excel, JMP, and other data analysis tools.

ACCURATE NORMALIZATION WITH SIMPLE WESTERN

A common use of Western blots and other immunoassays is to measure expression changes among target proteins. For this purpose, normalizing protein expression by a housekeeping protein can be misleading as many housekeeping proteins may change under conditions where their expression was previously assumed to be stable. This obfuscates protein expression results, and journals now strongly discourage the use of housekeeping or loading control proteins for protein normalization.[8] Instead normalizing the expression of target protein to the overall protein abundance in the sample is a more accurate way to measure changes in expression.

Simple Western addresses these issues by offering total protein normalization that allows for direct comparison between runs. Normalizing data by total protein detection may be performed by the chemiluminescence-based Total Protein Detection Assay, which is compatible with all Simple Western Size platforms, or by the fluorescence-based Protein Normalization on Jess. For more information on total protein detection with Simple Western, see Better Housekeeping with Jess: Total Protein Normalization, Total Protein: The Simple Western Way, and Using the Simple Western Total Protein Assay to Normalize Immunoassay Data in the Same Run.

MEETING RIGOROUS GRANT SUBMISSION GUIDELINES

Even before publication, Simple Western aids in experimental design and grant applications. There are now NIH enhancing reproducibility guidelines in place for grant submissions that require attachments for authentication of key biological and/or chemical resources.[13] In these guidelines, grant writers must describe methods to ensure the identity and validity of key biological and/or chemical resources used in the proposed studies. For example, antibodies must be validated, and their saturating limit of detection must be determined if they are to be used in the quantification of an antigen. Furthermore, sufficient biological and technical replicates are needed to ensure the reproducibility of experimental results and determination of experimental error. These aspects of method validation require running a large number of samples in a quantitative manner.

Compared with traditional Western blot, Simple Western's high-throughput capabilities facilitate running replicates and determining linear range of detection. Simple Western can run 25 samples in 3 hours or 96 samples overnight. This makes it easy to run the appropriate number of replicates required to generate concrete data, to establish the linear range of detection, and validate antibodies. With chemiluminescence detection, the linear dynamic range can reach up to 6 logs depending on the antigen. Taken together, Simple Western facilitates the design of rigorous studies that meet the NIH reproducibility guidelines for grant writers.

PUBLICATION-READY IMMUNOASSAY DATA

Simple Western fulfills the need for improved data integrity, rigor and transparency in traditional Western blotting workflows. As a manual multi-step process, traditional Western blotting is prone to pitfalls at nearly every step of the assay, from sample preparation, sample loading, electrophoretic transfer, blotting, imaging, and finally data analysis and publication. Simple Western alleviates the pitfalls associated with traditional Western blotting protocols by generating data that is transparent, rigorous, reproducible and not prone to misrepresentation. As a result, Simple Western makes it easier for researchers to publish their data with confidence that they are leading the way in generating reproducible, rigorous conclusions.

SIMPLE WESTERN RESOURCES

- Western Blotting Instrument Solutions
- Transferring Your Traditional Western Blot to Wes
- Simple Western FAQs
- How Can Simple Western Help You?

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