Best Practices for Publishing Simple Western Data

How-to Guide

Introduction

This guide outlines best practices for preparing and presenting protein analysis data using Simple Western™ Technology for publishing in peer-reviewed scientific journals. It focuses on data presentation, validation transparency, and figure integrity — without delving into instrument theory or operational detail.

When publishing Simple Western results, prioritize transparency, accuracy, and reproducibility. Figures and supplementary materials should provide sufficient information for peers to understand experimental design, validate the findings, and reproduce the assay if needed.

Citing Simple Western Technology
When referring to Simple Western instruments, include the name, catalog number, and vendor. For example, "Capillary-based western analysis was performed using the Simple Western™ Jess™ System (004-650, Bio-Techne)"

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Ensuring Validity of a Run

To ensure accurate results, verify that the fluorescent standards, biotinylated ladder, and samples have migrated correctly and are labeled appropriately. Proceed to analysis once the validity of a run has been confirmed. For more information visit the **Bio-Techne Academy** or refer to the **Compass for Simple Western User Guide**.

Standards Migration

Verify the migration of molecular weight standards within the run summary to ensure standards migrate as expected, allowing accurate molecular weight assignment (Figure 1).

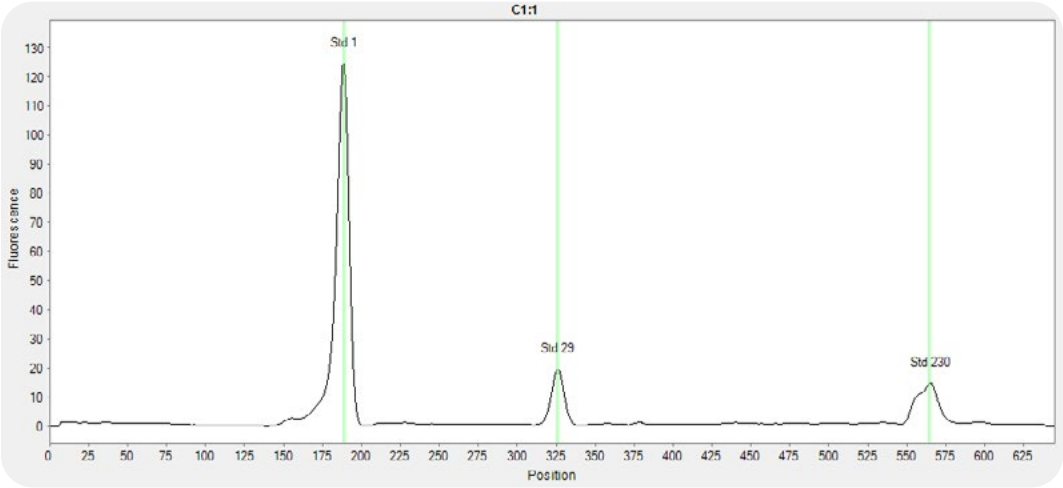


Figure 1. Check the electropherogram of the fluorescent standards has standard peaks labeled Std 1, Std 29 and Std 230 (for 12-230 kDa size assays), Std 57 and Std 280 (for 66-440 kDa size assays) or Std 1 and Std 26 (for 2-40 kDa size assays).

Biotinylated Ladder Peaks

Confirm all biotinylated ladder peaks are present, properly assigned, and all peaks align with expected ladder positions (Figure 2).

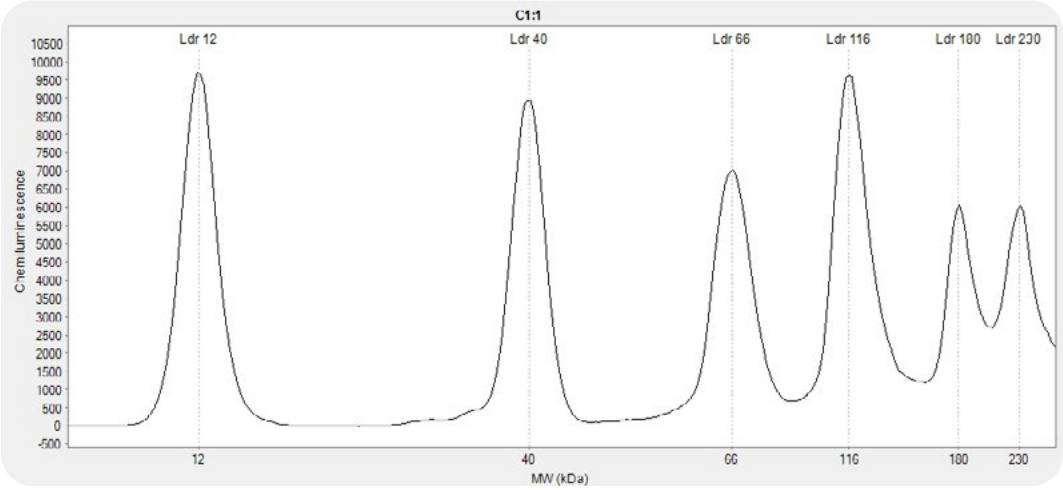


Figure 2. Verify the biotinylated ladder. Check that the electropherogram has either six ladder peaks (for 12-230 kDa size assays) or five ladder peaks (for 66-440 and 2-40 kDa size assays). In this example, the electropherogram has six peaks labeled Ldr 12, Ldr 40, Ldr 66, Ldr 116, Ldr 180 and Ldr 230.

Capillary View

Check the capillary view to ensure proper separation of samples and standards (Figure 3). Include the raw image view of the capillary in the supplementary or data repository for added transparency.

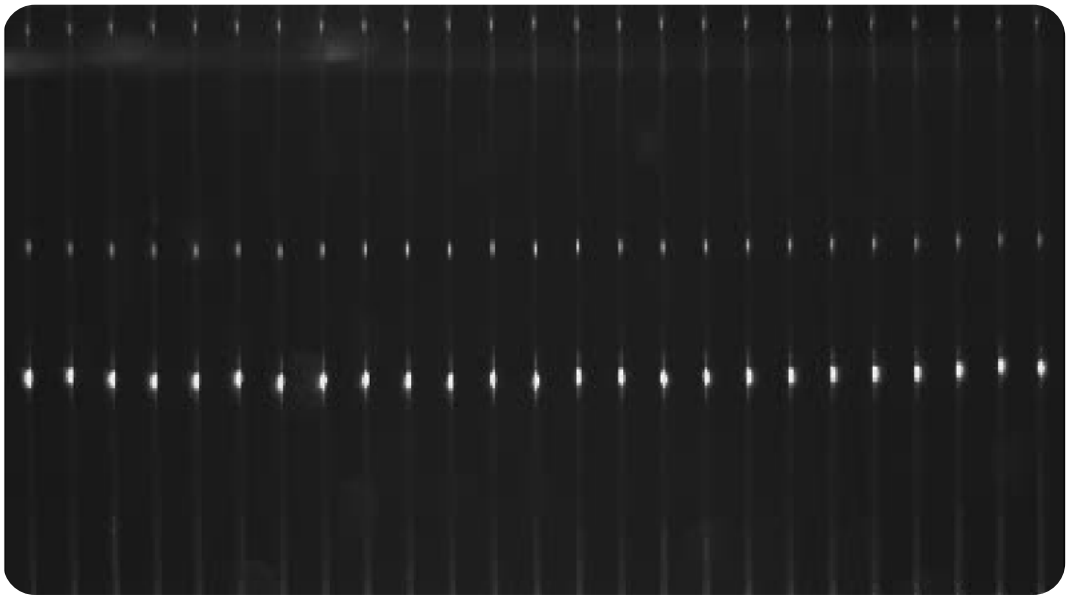
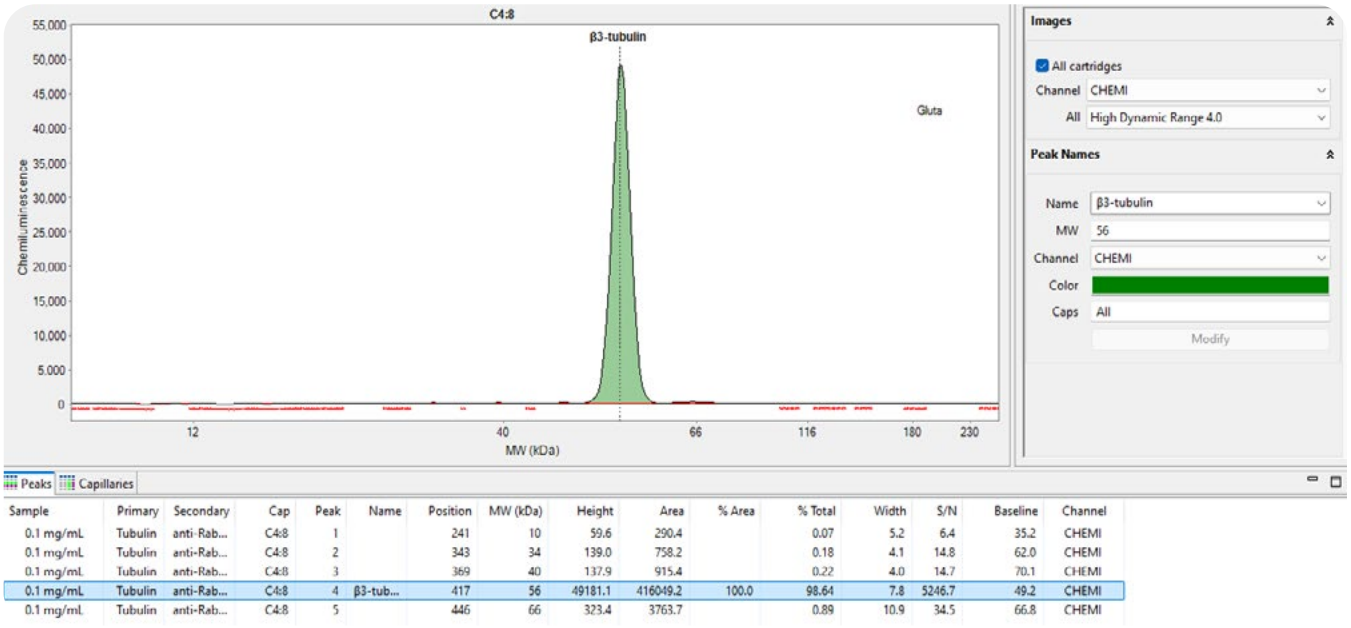


Figure 3. Verify the capillary image. All capillaries should have three fluorescent sizing standards for 12-230 kDa size assays or two fluorescent standards for 66-440 kDa and 2-40 kDa size assays.

Peak Fit and Baseline

Confirm accurate baseline fitting and peak area integration (Figure 4).



Sample	Primary	Secondary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	% Total	Width	S/N	Baseline	Channel
0.1 mg/mL	Tubulin	anti-Rab...	C4:8	1		241	10	59.6	290.4		0.07	5.2	6.4	35.2	CHEMI
0.1 mg/mL	Tubulin	anti-Rab...	C4:8	2		343	34	139.0	758.2		0.18	4.1	14.8	62.0	CHEMI
0.1 mg/mL	Tubulin	anti-Rab...	C4:8	3		369	40	137.9	915.4		0.22	4.0	14.7	70.1	CHEMI
0.1 mg/mL	Tubulin	anti-Rab...	C4:8	4	β3-tub...	417	56	49181.1	416049.2	100.0	98.64	7.8	5246.7	49.2	CHEMI
0.1 mg/mL	Tubulin	anti-Rab...	C4:8	5		446	66	323.4	3763.7		0.89	10.9	34.5	66.8	CHEMI

Figure 4. Verify baseline fitting and peak integration. Refer to the **Compass for Simple Western User Guide** for more information.

Preparing Figures for Publication

Use Compass for Simple Western for Figure Preparation

Figures should be prepared and annotated directly in Compass for Simple Western Software. With both electropherogram and lane view, users may annotate and export figures for publication. No additional image software is required for exporting figures.

Annotations

Clearly label detection channels, lanes, and peaks. Label each peak with sample and antibody name along with their respective dilutions. In the figure caption or materials and methods, state whether High Dynamic Range or a particular exposure time was used to visualize the data. Specify whether CHEMI, NIR, or IR detection was utilized.

Graph View

In graph view, your data are represented as an electropherogram with migration distance on the X-axis and signal intensity on the Y-axis. Prioritize graph view for publishing data for its superior resolution and quantitative detail, dynamic range e.g., insights into charge or size isoforms, cross-reactivity, or degradation products. To export graph view figures, right click the graph and click Copy (Figure 5).

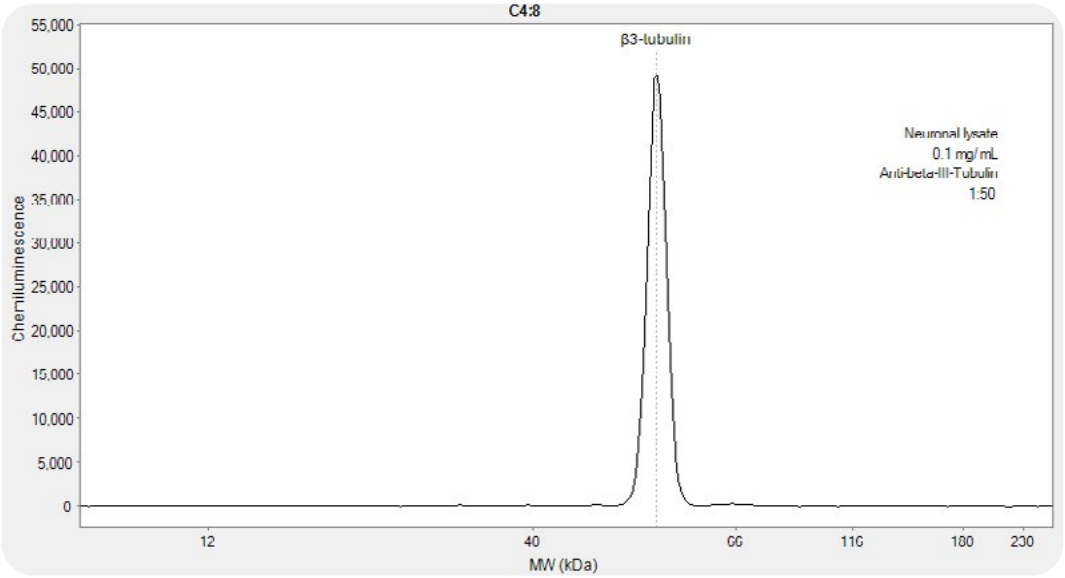


Figure 5. Export graph view figures for publication. Refer to the **Compass for Simple Western User Guide** for more information.

Lane View (Optional)

Traditional western blot users may be more accustomed to viewing their data as bands and lanes. Therefore, Compass also provides the option to view Simple Western data in a virtual lane view. Instead of peaks on an electropherogram, in lane view your target is presented as bands on an imaged membrane, just like a traditional western blot (Figure 6).

Optionally include the lane view but always accompany it with the graph view for clarity, either on the main figures or supplementary. Adjust the contrast in lane view by the same amount across compared runs to maintain data integrity. If the contrast is adjusted differently for different targets or different capillaries, this should be mentioned in the figure legend.

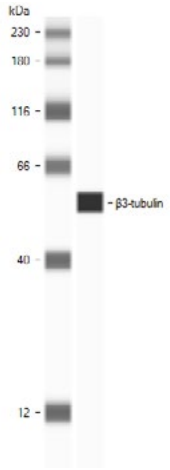


Figure 6. (Optional) Include lane view images to accompany graph view images (Figure 5). While graph view provides a more detailed view of your data, virtual lane view images may be more recognizable to traditional western blot users. Refer to the **Compass for Simple Western User Guide** for more information on exporting lane view figures.

Safe Lane Splicing

Because each capillary is a self-contained assay, the lanes can be easily reordered using just a drag and drop of the mouse. This eliminates having to rerun samples in a desired order and reduces risk of improper image manipulation such as splicing or duplication of lanes. Utilize Compass Software to rearrange lanes by simply dragging and dropping lanes into the desired order (Figure 7).

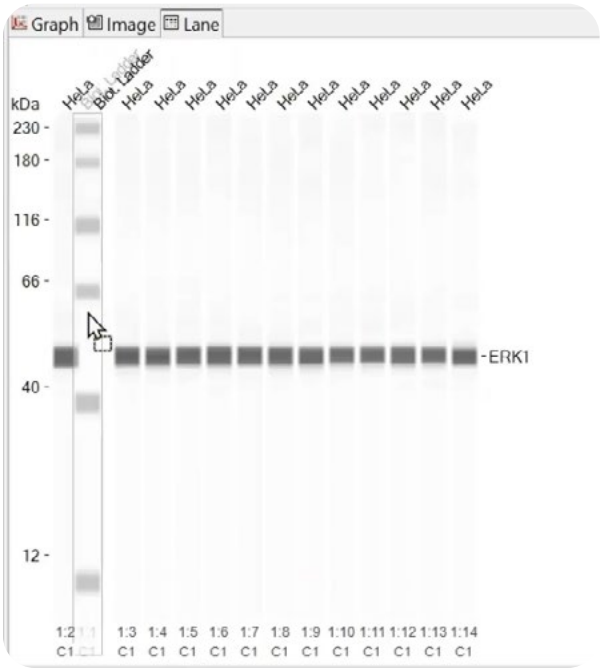


Figure 7. Drag and drop to rearrange lanes in the Compass Software lane view.

Quantitative Results Tables

In the supplementary or data repository, include the peak tables of corresponding capillary figures to showcase peak heights, signal-to-noise ratios (S/N), and baseline values (Figure 8).

Sample	Primary	Secondary	Cap	Peak	Name	Position	MW (kDa)	Height	Area	% Area	Corr. Area	Width	S/N	Baseline	Channel
Biot. Ladder	Antibod...	Streptavid...	P1:1	2	Ldr 40	323	40	18459.6	220504.5			11.2	5024.5	57.6	CHEMI
Biot. Ladder	Antibod...	Streptavid...	P1:1	3	Ldr 66	397	66	17130.1	147662.1			8.1	4662.6	63.5	CHEMI
Biot. Ladder	Antibod...	Streptavid...	P1:1	4	Ldr 116	443	116	12874.0	156780.1			11.4	3504.1	63.7	CHEMI
Biot. Ladder	Antibod...	Streptavid...	P1:1	5	Ldr 180	488	180	11010.0	131852.7			11.3	2996.8	60.8	CHEMI
Biot. Ladder	Antibod...	Streptavid...	P1:1	6	Ldr 230	510	230	11193.6	138716.1			11.6	3046.7	58.3	CHEMI
Reference	Primary ...	Secondary...	P1:6	1	Protein-x	362	54	225733.8	1663593.2	100.0	1673222.7	6.9	1371.3	2068.4	CHEMI
Reference	Primary ...	Secondary...	P1:7	1	Protein-x	361	54	236533.7	1803534.2	100.0	1723787.2	7.2	1731.5	1969.4	CHEMI
Reference	Primary ...	Secondary...	P1:8	1	Protein-x	362	54	227936.2	1661094.8	100.0	1634823.2	6.8	1971.4	2019.0	CHEMI
Reference	Primary ...	Secondary...	P1:9	1	Protein-x	362	54	186841.6	1389301.8	100.0	1513418.8	7.0	1378.4	2057.2	CHEMI
Treated 10 m...	Primary ...	Secondary...	P1:14	1	Protein-x	364	54	327069.9	2377831.9	100.0	2377831.9	6.8	1818.6	3608.1	CHEMI
Treated 10 m...	Primary ...	Secondary...	P1:15	1	Protein-x	365	54	328241.3	2405121.8	100.0	2466406.9	6.9	2055.8	3341.5	CHEMI
Treated 10 m...	Primary ...	Secondary...	P1:16	1	Protein-x	365	54	300181.8	2295604.3	100.0	2417879.0	7.2	1619.1	3387.4	CHEMI
Treated 10 m...	Primary ...	Secondary...	P1:17	1	Protein-x	366	55	324604.8	2419808.8	100.0	2396329.3	7.0	2525.4	3407.5	CHEMI

Figure 8. The quantitative results may be included in the supplement or provided by making the run files (.cbz) available (see below).

Writing Your Manuscript: Information to Include

Transparency is the key to quality results. Include the following information about your Simple Western experiments to facilitate peer-review and allow other researchers to reproduce your results.

Antibodies Used on Simple Western
In the Materials and Methods section, specifically list the antibodies used for Simple Western analysis. For both primary and secondary antibodies, it is critical to include:

- Name or target of the antibody
- Vendor and catalog number
- Dilution or concentration used

Additional assay information should be included where appropriate, such as:

- Sample type, e.g. Lysates of Jurkat human acute T cell leukemia cell line
- Sample preparation and concentration, e.g. Samples were prepared under reducing conditions using 1X Master Mix at 95 °C for 5 minutes, loaded at 0.2 mg/mL.
- Observed molecular weight within molecular weight range of separation, e.g. A specific band was detected for GAPDH at approximately 41 kDa using the 12-230 kDa Separation Module.

Source of Antibody Validation
Accompany your results with data supporting antibody validation on Simple Western. Antibody validation data can be included in the supplementary materials or referenced in the text if provided by a previous publication.

Protein Normalization
If applicable, include information on protein normalization. Whenever possible, normalize the expression of target proteins to overall protein abundance instead of using housekeeping proteins due to their instability. Ensure you test the linear range of detection for the normalization assay. For proper normalization, the primary antibody should have a linear response to lysate titration that overlaps with the linear total protein detection range.

Make Raw Data Available
Ensure raw data is available for consultation to promote transparency and reproducibility. With Simple Western, all raw data, including assay setup, run settings, and analysis settings are captured in self-contained run files that are easily shared or uploaded to data repositories. Ideally, these run files (.cbz) should be made available in the supplementary information or deposited in a public data repository.

Conclusion

Clear and transparent data presentation is essential for publishing high-quality Simple Western results. By verifying run validity, prioritizing graph view data, clearly annotating figures, fully reporting experimental details, and making raw data available, authors can support rigorous peer review and reproducibility.

When reported consistently, Simple Western data provides a robust and quantitative alternative to traditional western blotting, enabling confident interpretation and reproducible protein analysis across research applications.

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bio-techne® / Global Developer, Manufacturer, and Supplier of High-Quality Reagents, Analytical Instruments, and Precision Diagnostics.

INCLUDES R&D Systems™ Novus Biologicals™ Tocris Bioscience™ ProteinSimple™ Asuragen® ACD™ Lunaphore™