Wes Delivers Broader Dynamic Range Compared to Traditional Western

Broad dynamic range equals more data

An immunoassay with a large dynamic range lets you simultaneously see really high and really low signal at the same time. This means not needing to spend time either diluting or concentrating your samples to get your protein of interest in the detected dynamic range. Wes® has always been great at detecting really low levels of protein and now we've improved what you'll see on the high end, giving you at least one more log in dynamic range compared to a Traditional Western blot.

See more with Wes

We compared dynamic ranges on Wes and a Traditional Western blot by loading equal volumes and concentrations of samples on a SDS-PAGE gel and Wes plate.

HEK293 lysate was serially titrated 1:5 from 13.5 mg/mL down to 0.0002 mg/mL to run on both methods. Sample, prepared in Laemmli Sample Buffer with βME, was loaded onto a 4-20% SDS-Page gel, separated for 2 hours at 75, then transferred to a nitrocellulose membrane for 1 hour at 100 V. The membrane was immunoprobed with anti-GSK3α before imaging with a ProteinSimple FluorChem™ M (FCM) digital imager.

Wes samples were prepared in 5X Fluorescent Standard with DTT and loaded onto 12 – 230 kDa Wes plate. The plate was centrifuged for 5 minutes at 2500 rpm at room temperature then placed in Wes with a capillary cartridge. Proteins were separated in the capillary, captured to the wall using proprietary UV capture technology, then immunoprobed with anti-GSK3α and imaged using the High Dynamic Range (HDR) detection profile.

When we compared the Wes signal to a Traditional Western blot image taken on the FCM using the autoexposure feature (310 ms exposure time for this blot), we saw signal at lower concentrations of sample on Wes compared to the Traditional Western blot (**Figure 1**).

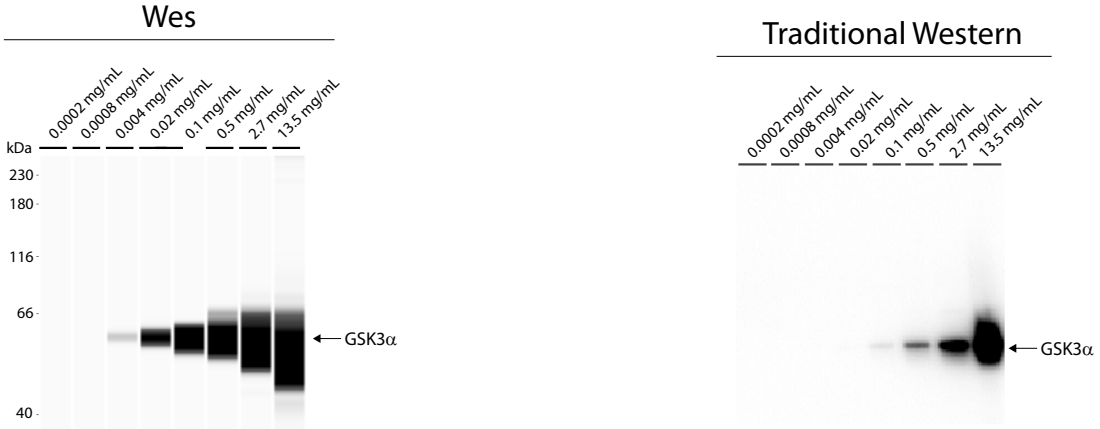

FIGURE 1. GSK3α was detected in HEK293 lysate serially titrated and run on Wes (left) and a Traditional Western blot (right). Lower signals were detected with Wes compared to the Traditional Western blot while both techniques detected signal at higher lysate concentrations.

When you maximize the lane view contrast on Compass for Simple Western, the software used to run and analyze Wes data, you can see signal all the way down to 0.0002 mg/mL (**Figure 2**). Using a longer exposure time on the FCM imager to try to see signal in the lower concentrated samples does let you see signal down to 0.02 mg/mL but the 13.5 mg/mL and 2.6 mg/mL sample signal is now over-saturated.

Graphing the signal from Wes and the Traditional Western blot image using the autoexposure feature captures the ≥1-log improvement in dynamic range (**Figure 3**).

Wes delivers a significantly wider dynamic range thanks to Wes' greater sensitivity and the use of the HDR detection profile. That means better detection and quantitation over a larger sample concentration range for you.

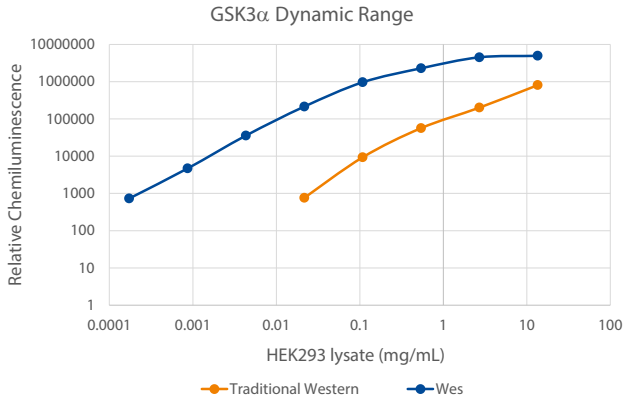

FIGURE 3. Signal comparison between the Wes and the Traditional Western blot demonstrates a 1.5-log dynamic range improvement with Wes.

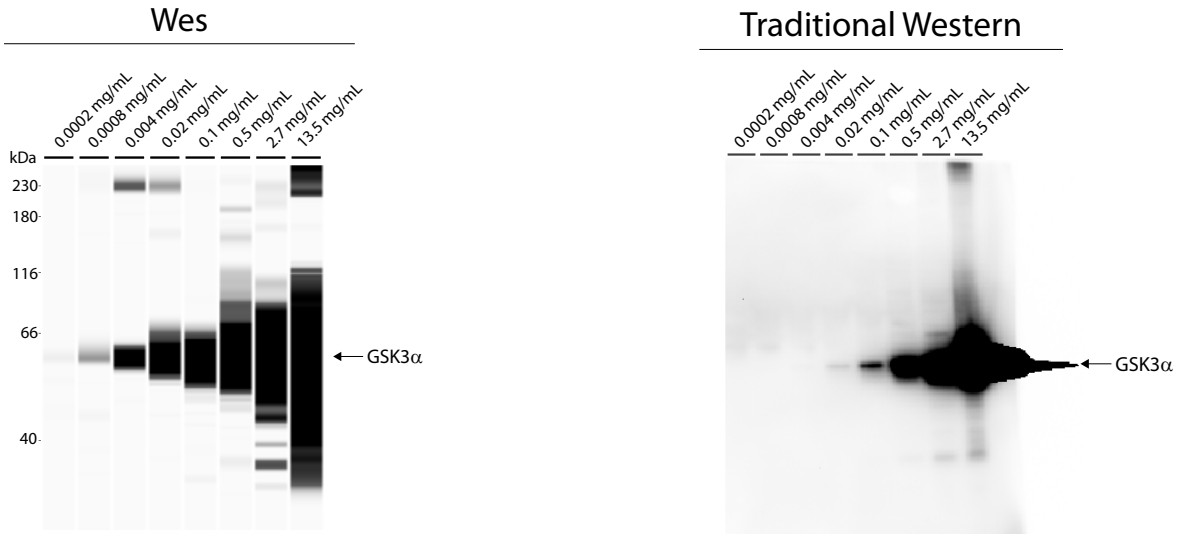

FIGURE 2. GSK3α signal is visualized in HEK293 lysate as low as 0.0002 mg/mL when the lane view in Compass for Simple Western contrast is increased (left). Increasing the Traditional Western blot exposure time to 30 seconds improves signal visualization at 0.02 mg/mL, but causes signal saturation at 13.5 mg/mL and 2.7 mg/mL (right).

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Toll-free: (888) 607-9692
Tel: (408) 510-5500
info@proteinsimple.com
proteinsimple.com

96-3005-00, Rev A