Simple Western Delivers Improved Serum Biomarker Sensitivity with No Secondary IgG Cross-Reactivity

Introduction

We're always looking for ways to make your research simpler and now we've made biomarker discovery in serum or plasma easier with Simple Western®. Researchers usually use ELISAs and traditional Western to investigate serum biomarkers. But traditional Westerns can be challenging when highly abundant proteins like IgG and/or albumin react with secondary antibodies used in immunoassays, even when sample is diluted prior to testing. Protein A or G agarose affinity columns can partially remove these highly abundant proteins and enrichment technology can dilute high abundance proteins and concentrate low abundance proteins, but they're time consuming and can change samples to the point where you risk losing the biomarker.

Enter the new Simple Western Biotin Detection Module (PN DM-004). This module detects biotinylated primary antibodies with a Secondary Streptavidin-HRP (SA-HRP) that's optimized for high sensitivity. You'll get information on MW, isoforms and multimers, and protein processing events without cross-reactivity from your conjugated secondary antibody. And it's a lot more sensitive compared to traditional Western.

In this technical note we show you how this new assays works and give you a couple examples comparing Simple Western performance with traditional Western.

Running a Streptavidin-HRP Assay

Running serum samples on Wes®, Sally Sue®, or Peggy Sue® is easy. Samples are prepared in 5X Master Mix, DTT, and 10X Sample Buffer so there's no more than 1.2 mg/mL of serum in the final sample and heat denature. Dilute the biotinylated primary antibody in Antibody Diluent 2, mix Luminol-S and Peroxide at a 1:1 ratio, and you're ready to set-up your plate (**Figure 1**). The ready-to-use Secondary SA-HRP is meant to replace the HRP-conjugated anti-species secondary antibody. Our assays are also so flexible that you can run cell lysate samples using an unconjugated primary/HRP-conjugated secondary and serum samples using a biotinylated primary/SA-HRP all in the same run.

Centrifuge the plate and place it in your Simple Western system. Then load the same assay you'd use for a standard immunoassay, click the **Start** button and you're done.

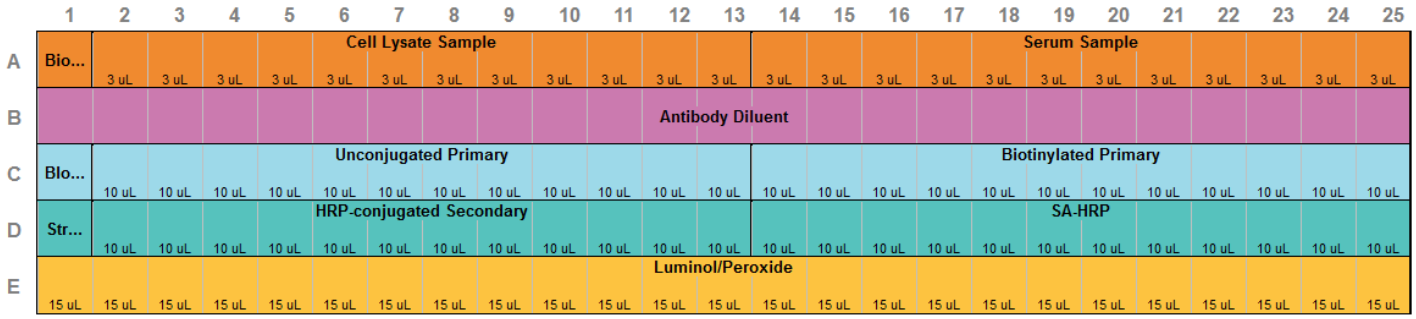

FIGURE 1. Schematic of an example Wes plate with the cell lysate assay (left) and serum sample lysate (right) on the same plate.

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No more secondary cross-reactivity with Simple Western

We first compared non-specific signal detected in human serum samples (Sigma H3667) using a HRP-conjugated secondary antibody and the Secondary SA-HRP detection reagent. Normal human serum sample was prepared and 3 µL pipetted into each well of a Wes plate. The primary antibody was replaced with 10 µL of Antibody Diluent 2 since we were only interested in the non-specific signal that comes from the secondary incubation. We then added either 10 µL of HRP-conjugated Anti-Mouse Secondary from ProteinSimple or 10 µL of Secondary SA-HRP into alternating wells and started a standard Wes 2-40 kDa assay.

Non-specific peaks were observed in the serum samples probed with Anti-Mouse Secondary (**Figure 2, blue trace**) when you use Compass for Simple Western to extend the x-axis out beyond 40 kDa. These peaks ran at 33 kDa and 56 kDa and most likely correspond to serum IgG light chain and heavy chain respectively cross-reacting with the anti-mouse HRP. These cross-reactive peaks were not detected when the serum was probed with Secondary SA-HRP (**Figure 2, orange trace**). While you don't see IgG cross-reactivity due to the secondary incubation interaction, this doesn't preclude cross-reaction of a primary antibody with a highly abundant protein in the serum, especially if your target of interest is in very low abundance.

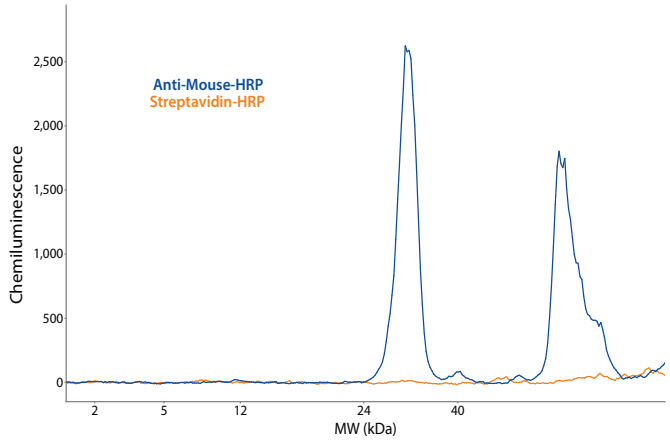

FIGURE 2. IgG cross reactivity seen in serum samples probed with Anti-Mouse HRP is not observed in serum samples probed with SA-HRP. The x-axis was expanded to view proteins beyond the 2-40 kDa matrix separation capacity in order to see both cross-reaction peaks.

Simple Western is more sensitive compared to traditional Western

We spiked different concentrations of recombinant IL-1α (R&D Systems 200-KA-010/CF) into the normal human serum (Sigma H3667) and ran samples side-by-side on Wes using a 2-40 kDa plate and on traditional Western using a 4-20% SDS-PAGE gel. Samples were probed using 3 µg/mL of IL-1α biotinylated antibody (R&D Systems BAF200) and then detected using the Secondary SA-HRP provided in the new Biotin Detection Module. Signal was observed on Simple Western with as little as 0.13 ng/mL recombinant IL-1α but at least 6.48 ng/mL of protein was required for detection with traditional Western (**Figure 3**). We then calculated the limit of detection (LOD) for both methods. Simple Western LOD was 0.085 ng/mL compared

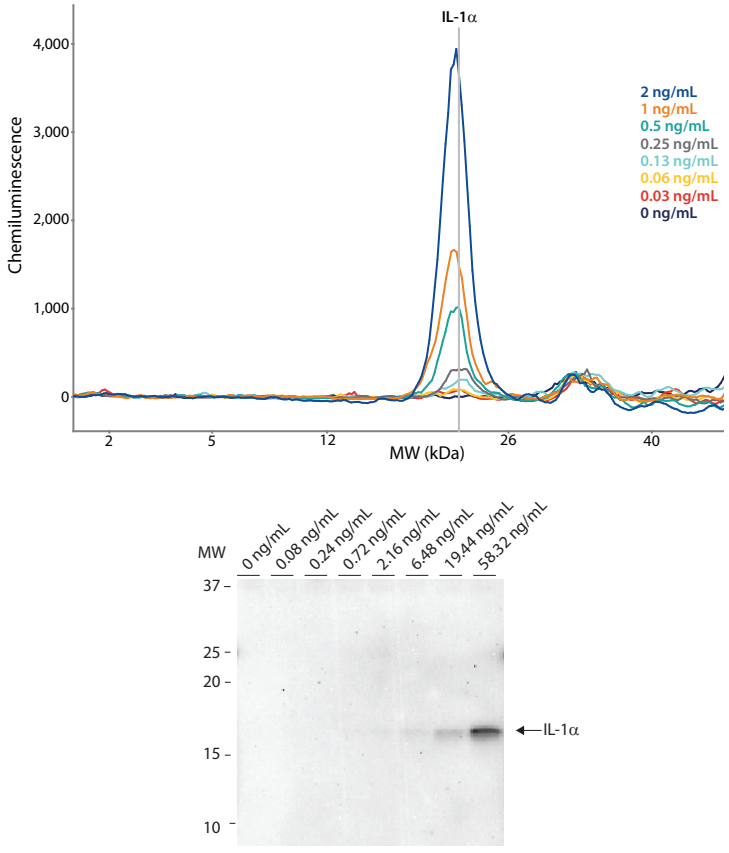

FIGURE 3. Wes (top) was more 56X more sensitive compared to traditional Western (bottom) when recombinant IL-1α spiked into human serum was detected using the Secondary SA-HRP reagent. Calculated limit of detection was 0.085 ng/mL on Wes and 4.9 ng/mL on traditional Western.

to 4.9 ng/mL for traditional Western – a more than 56X improvement in sensitivity.

We also compared side-by-side results for IL-6. Recombinant IL-6 (R&D Systems 208-IL-10/CF) was spiked into normal human serum and equivalent volume of sample were run on Wes using a 2-40 kDa plate and traditional Western using a 4-20% SDS-PAGE gel. Samples were probed using a 4 µg/mL biotinylated IL-6 antibody (R&D Systems BAF206) and detected using the Secondary SA-HRP detection reagent. Traditional Western required at least 1.62 ng/mL to observe a peak and had a calculated LOD of 0.8 ng/mL (**Figure 4**). Simple Western was more than 13X more sensitive with an observed peak at 0.13 ng/mL and a calculated LOD of 0.063 ng/mL.

Conclusion

The new Biotin Detection Modules now gives you isomer and molecular weight information for biomarkers in your serum and plasma without worrying about non-specific interactions between high abundance proteins, like IgG, with a secondary antibody. And increased sensitivity compared to traditional Westerns mean you'll be able to see things you couldn't before.

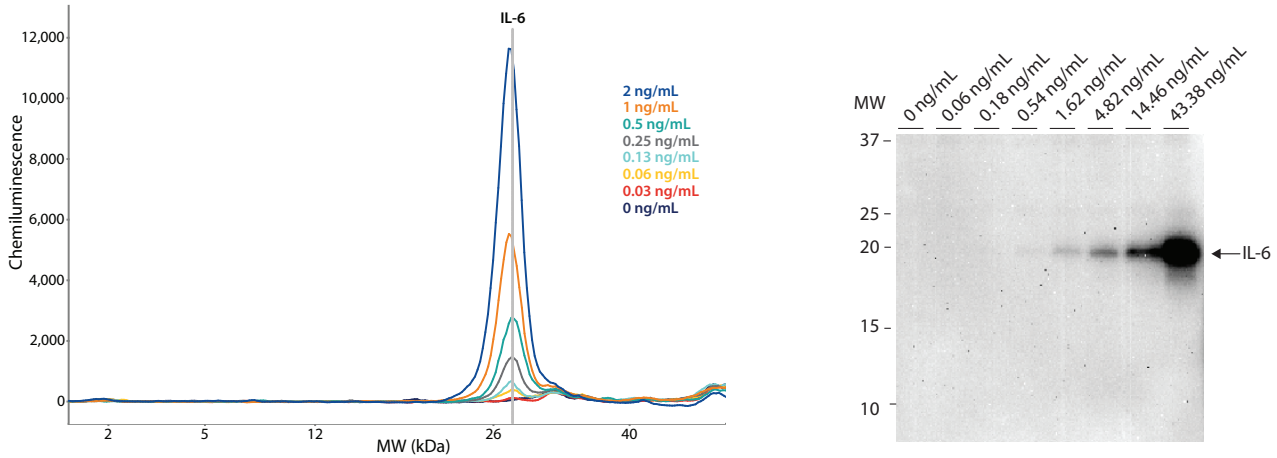

FIGURE 4. Wes (left) was more 13X more sensitive compared to Traditional Western (right) when recombinant IL-6 spiked into human serum was detected using the Secondary SA-HRP reagent. Calculated limit of detection was 0.063 ng/mL on Wes and 0.8 ng/mL on Traditional Western.

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