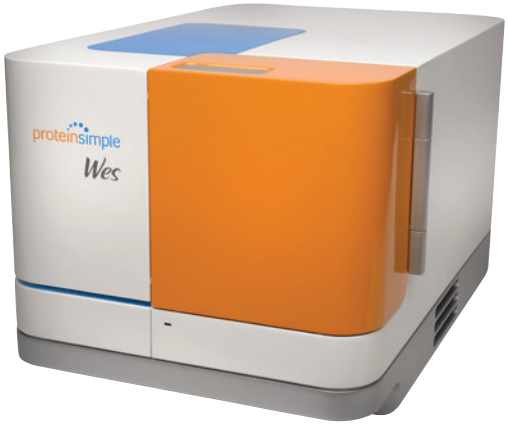
High Molecular Weight Protein Analysis Made Simple

Introduction



We all know analyzing a big protein using a traditional Western blot is easier said than done. First, the low percentage gel you have to use rips easily when you move it to the transfer apparatus. Next, there's the daunting task of optimizing transfer conditions to efficiently migrate your protein to the membrane. Plus it's also fairly common to have to transfer your protein overnight instead of the usual hour, increasing your time to results with a traditional Western blot from two days to three. Then at the end of those three long days, all you're left with is a method that'll give you sub-par reproducibility at best.

Well, you'll never need to worry about any of that again! Wes now analyzes proteins up to 440 kDa with the same Simple Western speed and data quality you've come to enjoy. And thanks to his proprietary capture chemistry, you'll never have to wonder if your proteins transferred efficiently either. To top that off, you can do all of this with just 30 minutes of hands-on-time and get results in less than three hours.

And while we'll primarily discuss Wes data in this application note, everything you'll see here can also be done on Sally Sue and Peggy Sue as well. So for those of you who really need a high-throughput solution — don't worry, that's available too!

Big Protein Capability Comes in a New Master Kit

Wes has always been great at analyzing proteins from 12 kDa to 230 kDa. But now he can analyze high molecular weight proteins too!

If you're already using Wes, Sally Sue or Peggy Sue for immunoassays in the 12–230 kDa range, we had to do a little reformulating for a few of the proteins in the 66–440 kDa range. The biotinylated ladder and fluorescent standards as well as the separation matrix and running buffer that go into the pre-filled plate are different in the 66–440 kDa and 12–230 kDa kits (**Table 1**).

Wes Simplifies the Workflow for Big Protein Analysis

Setting up an experiment on Wes has always been easy and that hasn't changed for the 66–440 kDa assay. All you have to do is pipet assay-specific reagents, like your samples and primary antibodies, into a pre-filled plate that already contains all the other reagents needed to run an experiment (**Figure 1**).

Next, just pick the assay type in Compass v2.6 and up (**Figure 2**), the software used to acquire and analyze data, set up your run conditions, hit start, and walk away!

INSTRUMENT	CONSUMABLE	12–230 KDA REAGENT	66–440 KDA REAGENT
Wes	Ready-to-Use Plate	Wes Pre-filled Plate	Wes 66–440 kDa Pre-filled Plate
Wes, Sally Sue, Peggy Sue	Fluorescent Standards	Standard Pack 1 (29 kDa system control)	Standard Pack 3 (90 kDa system control)
		Standard Pack 2 (180 kDa system control)	Standard Pack 4 (200 kDa system control)
Sally Sue, Peggy Sue	Separation Matrix	Separation Matrix 2	Separation Matrix 3
	Running Buffer	Split Running Buffer 2	Split Running Buffer 3

TABLE 1. Differences in reagents between the 12–230 kDa and 66–440 kDa Master Kits.

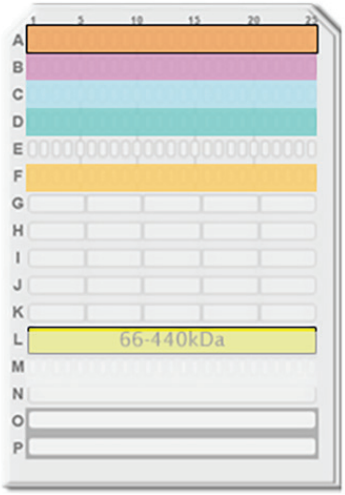


FIGURE 1. Wes 66–440 kDa pre-filled plate. Samples and ladder go in Row A (orange), blocking buffer in Row B (purple), primary antibody in Row C (light blue), secondary antibody in Row D (teal), Luminol/ Peroxide in Row E (gold), and Wash buffer goes in rows G–I. All remaining reagents are pre-filled for your convenience in rows L–P.

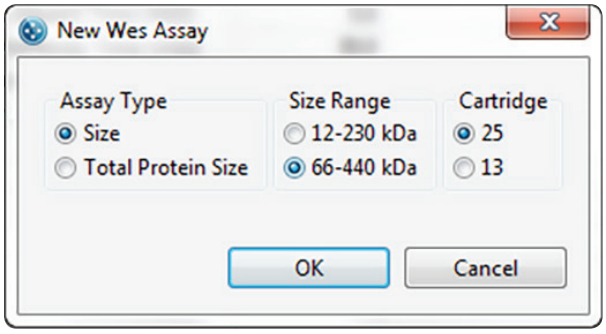


FIGURE 2. Choosing the Wes assay type in Compass.

If you're already a Wes user, the workflow for the 66–440 kDa assay is exactly the same so it's really easy to switch between running the 12–230 kDa assay and the 66–440 kDa assay. Just make sure you use the right reagents and right Compass protocol for the assay you want to run.

So what does the data look like? Let's take a look.

Assay Comparison for 66–230 kDa Proteins

As you may have already noticed, there's an overlapping molecular weight region between the 12–230 kDa assay and the 66–440 kDa assay. So we compared a panel of proteins with molecular weights between 66 and 230 kDa on Wes using both the 12–230 kDa and 66–440 kDa pre-filled plates (**Figure 3**).

Even though they use different separation matrices, both assays provide molecular weight standards spanning the 66–230 kDa region. So as expected, Compass reported similar apparent molecular weights for proteins from 66–230 kDa on both plates (**Table 2**).

So which kit should you use if your protein is 66–230 kDa? We recommend using the 66–440 kDa kit for proteins larger than 200 kDa for more accurate sizing. But if your protein is smaller than 200 kDa, either kit will give you great data.

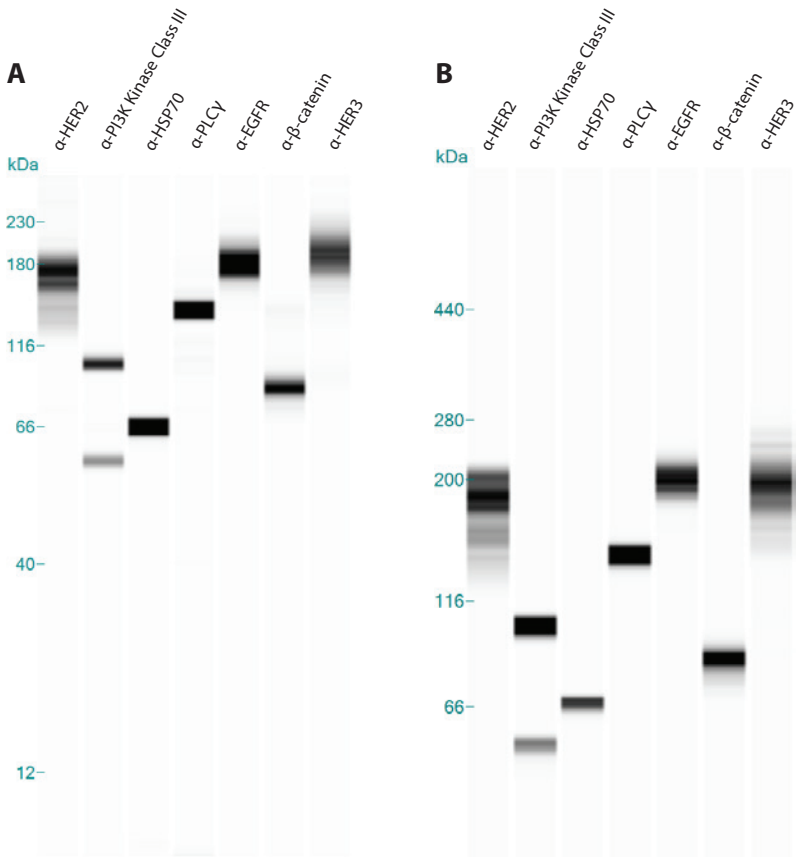


FIGURE 3. Panel of proteins run using both a 12–230 kDa (A) and a 66–440 kDa (B) pre-filled plate. We used A431 whole cell lysates for all assays except HER3 where MCF7 lysates were used. 1.0 µg/µL lysates were run for HER2, and HER3 while 0.01 µg/µL were used for EGFR and β-catenin. 0.25 µg/µL lysates were used for all other assays. All samples were denatured at 95 °C for 5 minutes except HER2 and HER3 where lysates were denatured at 70 °C for 10 minutes.

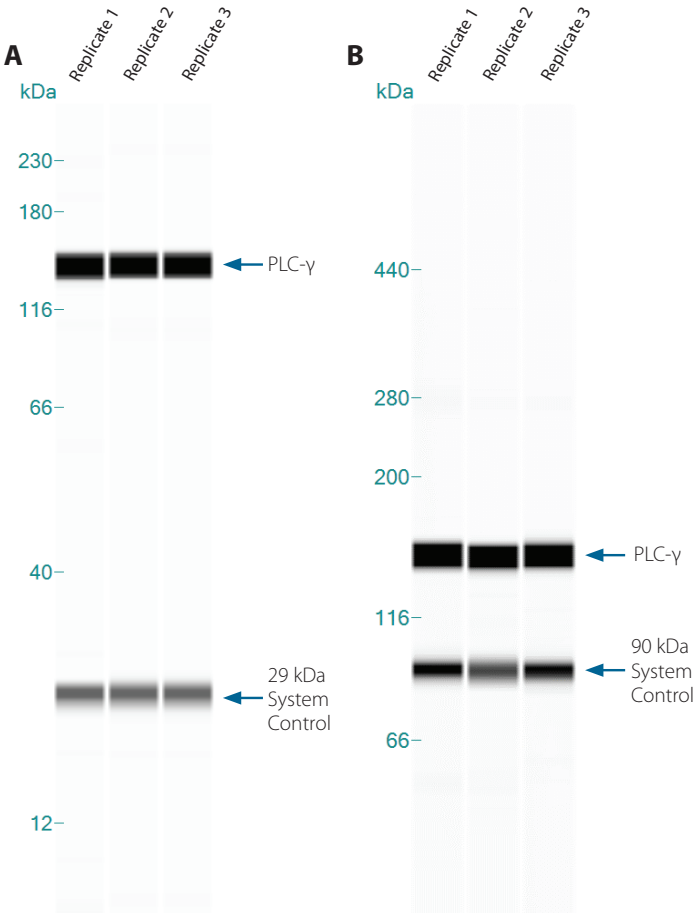
PROTEIN	12–230 KDA ASSAY	66–440 KDA ASSAY
HSP70	66 kDa	68 kDa
β-catenin	90 kDa	88 kDa
PI3K	59 kDa and 105 kDa	48 kDa and 104 kDa
PLC-γ	144 kDa	147 kDa
EGFR	180 kDa	200 kDa
HER2	172 kDa	189 kDa
HER3	192 kDa	218 kDa

TABLE 2. Comparison of the apparent molecular weight reported by Compass using both 12–230 kDa and 66–440 kDa prefilled plates.

To confirm you can get the same great data precision with both assays on Wes, we ran A431 lysates in triplicate capillaries and immunoprobed for PLC-γ. We then normalized the PLC-γ peak area to either the 29 kDa system control or 90 kDa system control for the 12–230 kDa and the 66–440 kDa pre-filled plate respectively (**Figure 4**). As expected, both matrices generated extremely reproducible results on Wes with CVs ≤3.8%. So, you'll get the same great reproducibility on Wes regardless if you use the 12–230 kDa or the 66–440 kDa Master Kit.

Analyzing Large Proteins

Next we ran a panel of high molecular weight proteins on Wes to show the extended sizing range the 66–440 kDa assay delivers. Six proteins with molecular weights greater than 230 kDa were run using the 66–440 kDa pre-filled plate (**Figure 5**). It took only 30 minutes of hands-on time to set up the plate and the time to results was a little under three hours. We used default assay conditions to generate the data but developing an assay using the 66–440 kDa pre-filled plate is just as simple and straightforward as it is



	12–230 KDA ASSAY	66–440 KDA ASSAY
% CV	3.5%	3.8%

FIGURE 4. A431 lysates at 0.25 µg/µL were run in triplicate capillaries and immunoprobed for PLC-γ and the system control to assess system reproducibility. Samples were run on both the 12–230 kDa (A) and 66–440 kDa (B) pre-filled plates. All CVs were ≤3.8%.

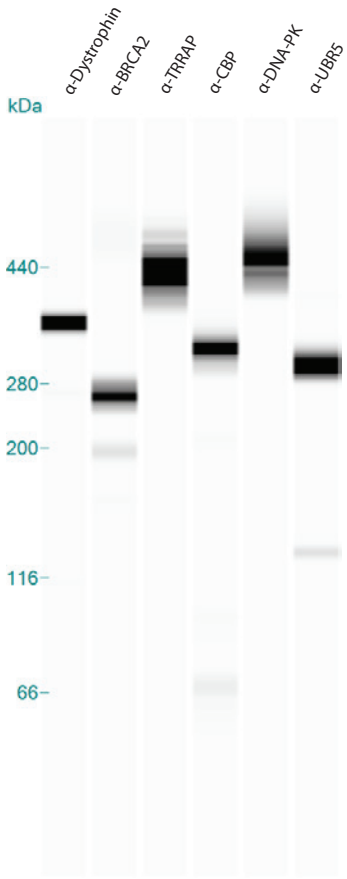
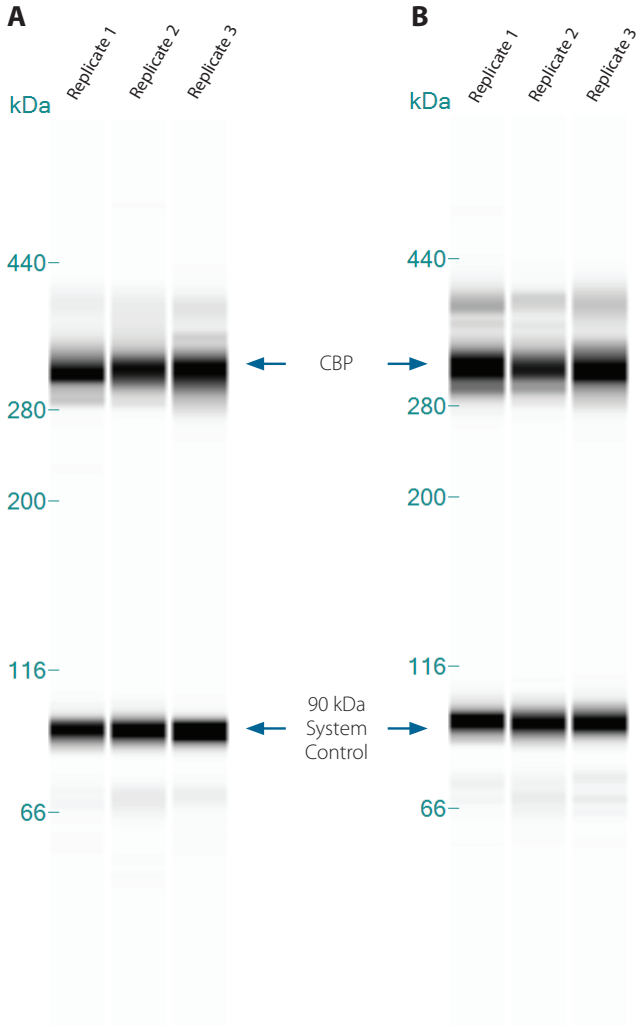


FIGURE 5. Panel of large proteins run with the 66–440 kDa pre-filled plate on Wes. We used 0.2 µg/µL of A10 lysate to detect dystrophin, 2 µg/µL of A431 lysate to detect BRCA2, 1.0 µg/µL of A431 lysate to detect TRRAP, 0.25 µg/µL of K562 lysate to detect CBP, and 1.0 µg/µL of K562 to detect DNA-PK and UBR5. All samples were denatured at 95 °C for 5 minutes.



	RUN 1	RUN 2	INTER-ASSAY (RUN 1 AND RUN 2)
% CV	8.6%	7.2%	7.1%

FIGURE 6. K562 lysates at 0.25 µg/µL were run in triplicate capillaries and immunoprobed for CBP and the system control to assess system reproducibility. Run 1 (A) and Run 2 (B) triplicates were run on two different days on Wes. Intra-assay CVs were ≤8.6% with an inter-assay CV of 7.1%.

with the 12–230 kDa plate[1]. And since Wes is a transfer-free system, we were able to quickly generate data for all the large proteins without a lot of optimization.

Transferring proteins to the membrane is a notorious source of variability for traditional Western blots, especially for larger proteins. So we expect CVs to be <15% as an additional benefit of being transfer-free. To demonstrate this, we ran the CBP assay on Wes in triplicate capillaries on two different days and normalized the CBP peak area to the 90 kDa system control (**Figure 6**). CVs were less than 8.6% for the first run and 7.2% for the second, and the inter-assay CV was an amazing 7.1%!

Conclusion

Never want to face a ripped gel, overnight transfer, or sky-high CVs for your high molecular weight proteins again? Done! With the new 66–440 kDa Master Kit, you can now enjoy all Wes has to offer for a greater range of proteins. You'll get the same exceptional Simple Western data with none of the hassle you'd normally have to deal with when analyzing big proteins. Our intra-assay CVs were all less than 8.6% and the inter-assay CV across multiple runs for CPB, a protein around 300 kDa, was 7.1%! Now there's finally a gel-free, hands-free, transfer-free way for you to analyze your high molecular weight proteins.

References

1. Easy transfer of your traditional Western Blot to Wes, *ProteinSimple Application Note.*

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