Molecular weight determination by electrophoresis of SDS-denatured proteins

There is a good reason why it is called apparent molecular weight

SDS-PAGE and Simple Western separate proteins based on the amount of SDS bound to the protein during denaturation. The correlation between motility and molecular weight (MW) is based on the uniform binding of negatively charged SDS to the protein (1.4g SDS/g Protein) which results in a constant charge-to-mass ratio[1,2]. In reality, the amount of SDS bound is anything but constant and it's affected by many factors. Among them are:

- Matrix or gel type used
- Running buffers (Tris-HCl, Bis-Tris, Borate, etc.)
- Characteristics of the target protein such as:
  - -hydrophobicity/hydrophilicity
  - -acidic and basic residues
  - -proline-rich regions
  - -splice variants
  - -multimers
  - -post translational cleavage and modifications like phosphorylation and glycosylation

In addition, some proteins can have secondary interactions with the separation matrix that affect their motility during separation. SDS-PAGE and Simple Western do not use the same separation matrix so you should expect these secondary interactions to differ.

Last but not least, the ladder used for SDS-PAGE Western can affect the resulting apparent MW[3]. In **Figure 1** you can see the variability of the MW calculated for endogenous Smac/DIABLO in HeLa based on molecular weight ladders from nine different vendors (LD1 to LD9) run on the same SDS-PAGE traditional Western experiment.

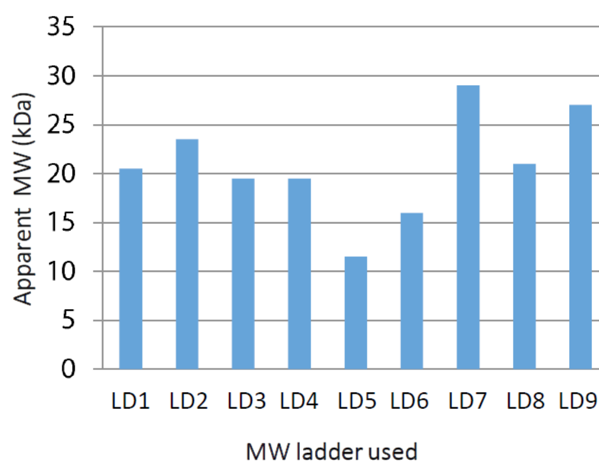


Figure 1: MW for Smac/DIABLO in traditional Western using ladders from different vendors.

In a nutshell: apparent MW calculated from SDS-PAGE or Simple Western separation will differ from the MW based on sequence or Mass Spec. One example for significant divergence between Apparent MW and MW based on sequence is PRAS40. This protein has a MW of 27,000g/mole by sequence, but runs around 40 kDa in SDS-PAGE Western (Example 1 below).

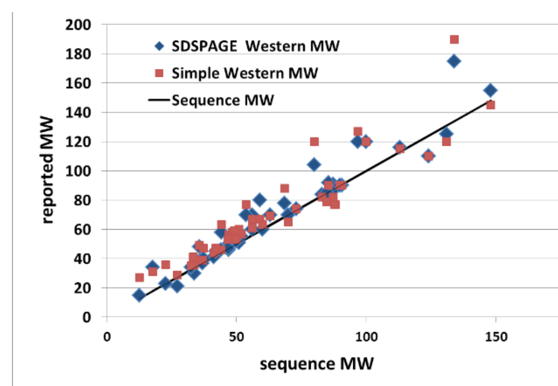


Figure 2: Correlation between apparent MW calculated by SDS-PAGE Western (blue) or Simple Western (red) and sequence MW.

Despite system differences for specific proteins, both SDS-PAGE and Simple Western show good correlation of apparent MW and MW calculated by sequence for most proteins as shown in **Figure 2** for over 70 different proteins run in Simple AND SDS-PAGE Western. Especially for Simple Western, which

returns an actual value for each peak this apparent MW value is very reproducible within the Simple Western platform.

How is the apparent MW calculated for Simple Western? So you don't have to work extra, Compass automatically creates a point to point fitted curve of the entered MW and measured position of each peak in the MW ladder. This curve is used to convert the position of each sample peak to the target's reported molecular weight.

Which MW should Simple Western match?

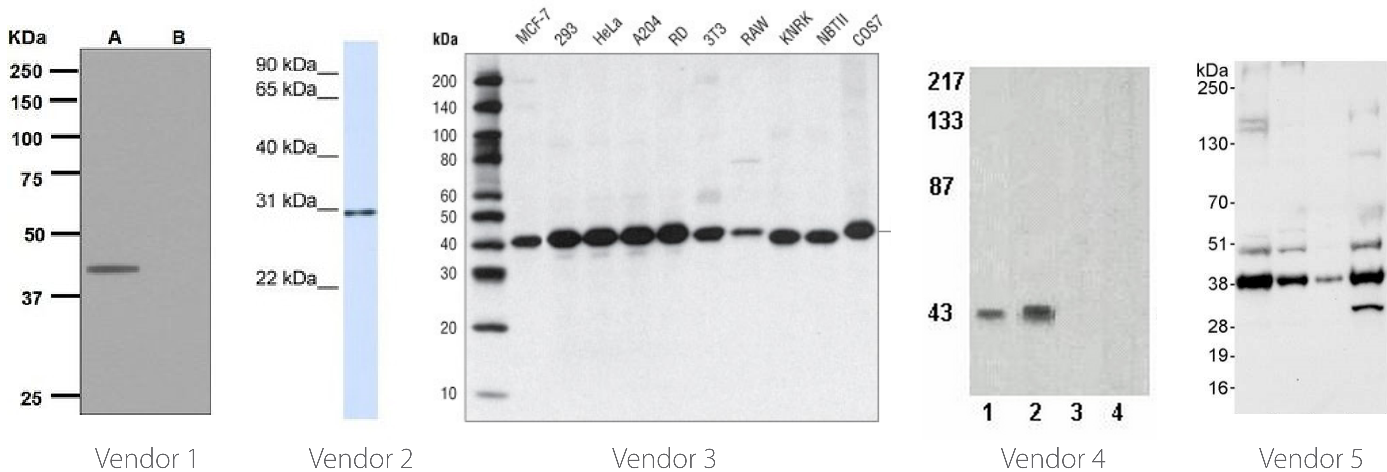
Now that you are using Simple Western you may see a peak, but not at exactly the expected apparent MW you obtained by running the same sample and using the same antibody by SDS-PAGE Western. And since the SDS-PAGE technique is still considered the gold standard and it's used to validate antibodies, you consider that apparent MW to be the correct one and expect to reproduce it with Simple Western. So, that means that every time you run a SDS-PAGE Western you should get the apparent MW for a particular target that is equal to the reported MW by the antibody vendor right? Let's take a look at the following examples.

EXAMPLE 1:

Target: PRAS40

MW based on its sequence: 27.38 kDa

Apparent molecular weight as determined by its detection with antibodies from five different vendors:



Vendor 1 Vendor 2 Vendor 3 Vendor 4 Vendor 5

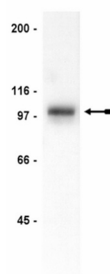
EXAMPLE 2:

Target: Gab1

Molecular weight sequence: 76.6 kDa

Ab vendor says: 115 kDa

Apparent MW: 98 kDa



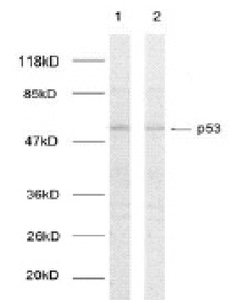
EXAMPLE 3:

Target: p53

Molecular weight by sequence:

Ab vendor says: 43 kDa

Apparent MW: 53 kDa



How can I be sure I am looking at right peak or band?

So how do you make sure the peak you see with Simple Western is your protein of interest? The same way you confirm in a SDS-PAGE Western:

1. You observe a clean, single peak in the electropherogram view
2. You observe a peak at the same MW as in a validation experiment using the same system (check our antibody database and the R&D Systems/Novus websites for lots of examples)
3. You observe the peak with your positive control but not with your negative control
4. Multiple antibodies against the target result in a peak at the same MW (the MW reproducibility in Simple Western is exquisite and quantifiable)

References:

1. A. Rath et al. PNAS 2009, Vol. 106, Number 6, 1760-1765
2. L. M. Hjemel et al. Electrophoresis 1981, Vol 2, Issue 1: 1-11
3. M. Sallantin et al. Electrophoresis 1990, Vol 11, Issue 1: 34-36

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