

Checklist for Quantitative Western Blots

Getting quantitative data from your western blot takes more than just a fluorescent or chemiluminescent secondary and a digital imager. You need to consider your sample prep, your normalization method, and the linearity of your signal. To help keep your quantitative western blot workflows on track, we've created this checklist to ensure your western blot quantitation is as accurate as it can be.

Consistency is the key to quantitative western blots

- Have you treated your samples consistently, ensuring cells and tissues are thoroughly lysed, immediately placed on ice, and/or been treated with appropriate reagents such as protease inhibitors?
- Have you loaded similar amounts of total protein in each lane (within a factor of 2- to 5-fold)?
- Are you using a robust and consistent western blotting protocol (reduce variability by using the same reagents for each blot or implementing lot-to-lot controls, and keeping your transfer conditions and incubation and wash times the same)?
- Are you normalizing to total protein?
- Have you validated the specificity of your antibodies?
- Have you verified that your system is linear? The linearity of your signal can be affected by multiple factors. Here are steps you can take to ensure linearity and improve the accuracy of your quantitation:
 - Are the highest and lowest amounts of your protein-of-interest in the linear range (note that you need to verify linearity with each protein-antibody pair)?
 - Are you saturating your membrane?
 - Have you optimized your primary and secondary antibody dilutions? Titrating your antibody against your protein-of-interest can reveal an antibody dilution that delivers the widest dynamic range.
 - Have you optimized your image acquisition time to ensure you are in the linear range of your imager?



cSeries™ Imagers

Blending powerful performance with a budget-friendly price, cSeries Imagers excel at quantitative western blot imaging and a whole lot more.

See the many cSeries configurations and get a quote at azurebiosystems.com/cseries-imaging-systems



Sapphire™ Biomolecular Imagers

For the ultimate in quantitative western blot imaging performance, turn to the Sapphire Biomolecular Imager.

- RGB fluorescence
- NIR fluorescence
- Chemiluminescence
- Phosphorimaging

Find out just how much performance you can pack in a hybrid laser scanner/imager at azurebiosystems.com/sapphire



AzureRed Total Protein Stain

Easily stain total protein for the most accurate western blot normalization.

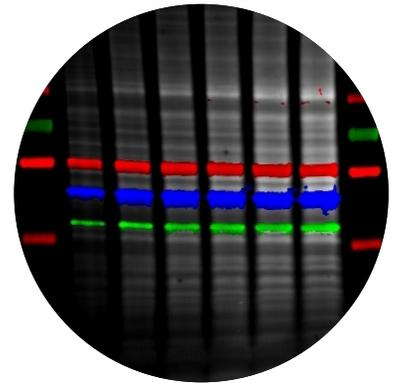
Catalog Number AC2124

Western Blot Quick Quiz

What's the best, most efficient way to do **total protein normalization** of NIR blots?

- ~~X~~. Probe for two proteins, image, strip the blot, restain for total protein, and re-image
- ~~X~~. Run two blots

Q. With **Sapphire™**. Run one blot, probe for two proteins, stain for total protein, and image



Use the **Sapphire NIR-Q** to probe up to 2 proteins and the **Sapphire RGBNIR** to probe up to 3 proteins.



Learn more at azurebiosystems.com/nirq

