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?

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