

**INTRODUCTION**

Although western blotting methods have been in practice for three decades, only recently have peer-reviewed and journal-quality articles surfaced on this topic. They now significantly demonstrate the validity of the methods by which they quantify their western blot (WB) results. Such demonstration work is needed to build a better understanding of why western blot method inaccuracies and other inherent experimental variability that would presumably be unavoidable is being addressed. Protein normalization is a method by which the amount of a target protein can be determined relative to a reference, often a housekeeping protein (HKP) like the α-actin, β-actin, or glyceraldehyde 3-phosphate dehydrogenase (GAPDH). An HKP is chosen and used as a reference with the assumption that the level of this HKP would be directly proportional to the total amount of protein in the samples being changed. Changes in a sample’s target protein levels arising from designed experimental perturbations are then expressed as ratios to the levels of the measured HKP. Unfortunately, the assumptions underlying the use of HKPs for protein normalization of western blots are not always met: a range of inconsistencies, inaccuracies, and missing conclusions.

**RESULTS**

The capacity of the Normalization Protein Labeling Reagent to serve as a means for performing TPP was assessed in various cell lines and compared to quantification results obtained using HKPs. For each protein, the Normalization Protein Labeling Reagent demonstrated that at least demonstrably a linear relationship between protein load and measured signal intensity.

**ACKNOWLEDGEMENTS**

We thank Greg K linen for pioneering the use of the HKP IVT cell lines used in this study. We acknowledge the support of Brian Webb and Thermo Fisher Scientific. We are also grateful to Alrik Tommer and David Piper for guidance and manuscript review.

**TRADEMARKS/LICENSEING**

© 2019 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc, and its subsidiaries unless otherwise specified. For Research Use Only. Not for use in diagnostic procedures.