Defining the New Normal in Quantitative Western Blot Data

By Geetha Yadav and Kenneth Oh

Do you know that journals like the JBC strongly caution against the use of housekeeping proteins for normalization? Most journals now demand rigorous standards for presentation of western blotting data. While housekeeping proteins (HKP) have traditionally been used for decades, the problems with their usage have come to the fore only recently. More validation is required for presenting data using HKP. Normalization using total proteins present in the sample has gained significant traction and has proved to be a reliable method for normalization of western blots. Here we illustrate why the total protein normalization (TPN) method is superior and the way to go for western blotting.

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DEFINING THE NEW NORMAL
IN QUANTITATIVE WESTERN BLOT DATA

HOW DO I ACCURATELY QUANTITATE THE CHANGES IN MY PROTEINS?

OPTION A — HKP — NORMALIZE TO HOUSEKEEPING PROTEIN (HKP)

Normalizing with HKP: like trying to hit a moving target.

The expression level of HKPs can change due to:

- Experimental conditions
- Developmental changes
- Post-transcriptional regulation
- Differences in cell, tissue age, and type
- Stages in cell cycle

OPTION B — TPN — USE TOTAL PROTEIN NORMALIZATION (TPN)

PREFERRED METHOD

Normalizing with total protein: multiple arrows covering the entire target area.

TPN takes into account:

- The intensity of all proteins in the lane
- Sample loading variations
- Variations during electrophoresis
- Variations during transfer

TPN PROVIDES ACCURATE QUANTITATION OF TARGET PROTEINS.

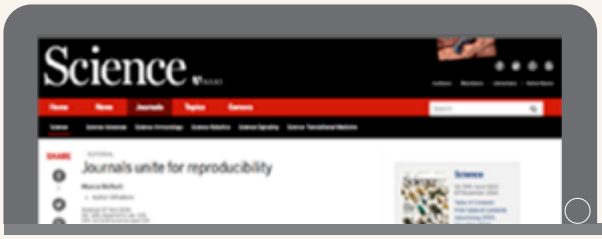
Total protein normalization is the most reliable method of reporting quantitative western blot data.

TPN is the preferred method of normalization for:

- Stem cell research
- Neuroscience research
- Alzheimer's research
- Developmental biology
- Aging studies

SEVERAL GROUPS HAVE EXPRESSED CONCERNS REGARDING WESTERN BLOT DATA NORMALIZATION:

- Nature
- Science
- NIH workshops and meetings



JOURNALS LIKE JOURNAL OF BIOLOGICAL CHEMISTRY PREFER TPN FOR SUBMITTING WESTERN BLOT DATA FOR PUBLICATION.

JBC editors recommend TPN for normalizing western blots.

> It is typically better to normalize western blots using total protein loading as the denominator.

> We prefer that signal intensities are normalized to total protein by staining membranes with Coomassie Blue, Ponceau S, or other protein stains, and we strongly caution against the use of housekeeping proteins for normalization, unless there is clear demonstration that expression of the housekeeping protein is unaffected by experimental treatments.

Fosang AJ and Colbran RJ (2015). Transparency is the key to quality. J Biol Chem 290, 29,692–29,694.

http://www.jbc.org/site/misc/ifora.xhtml

BIO-RAD'S V3 Western Workflow™ INCORPORATES TPN FOR NORMALIZATION OF WESTERN BLOT DATA.

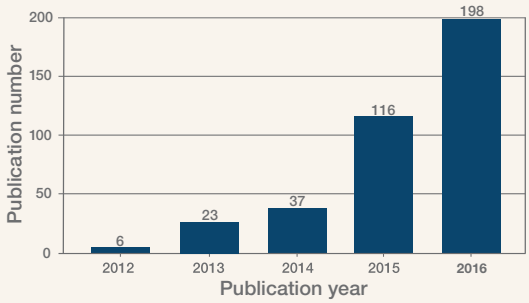
V3 Western Workflow includes:

- Stain-free visualization of total proteins on gel after electrophoresis
- Quantitation of total proteins in a lane after transfer
- Normalization of bands to total proteins by specialized software

Separate Proteins 1 → Visualize Separation 2 → Transfer Proteins 3 → Verify Transfer 4 → Validate Western Blot 5

MORE AND MORE SCIENTISTS USE V3 Western Workflow AND TPN FOR DATA NORMALIZATION.

The number of papers using the V3 Western Workflow and TPN has been consistently increasing over the years.



TOTAL PROTEIN NORMALIZATION IS THE IDEAL WAY FOR ACCURATE WESTERN BLOT NORMALIZATION.

PREFERRED ★ METHOD

Learn more about TPN and Bio-Rad's V3 Western Workflow.

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