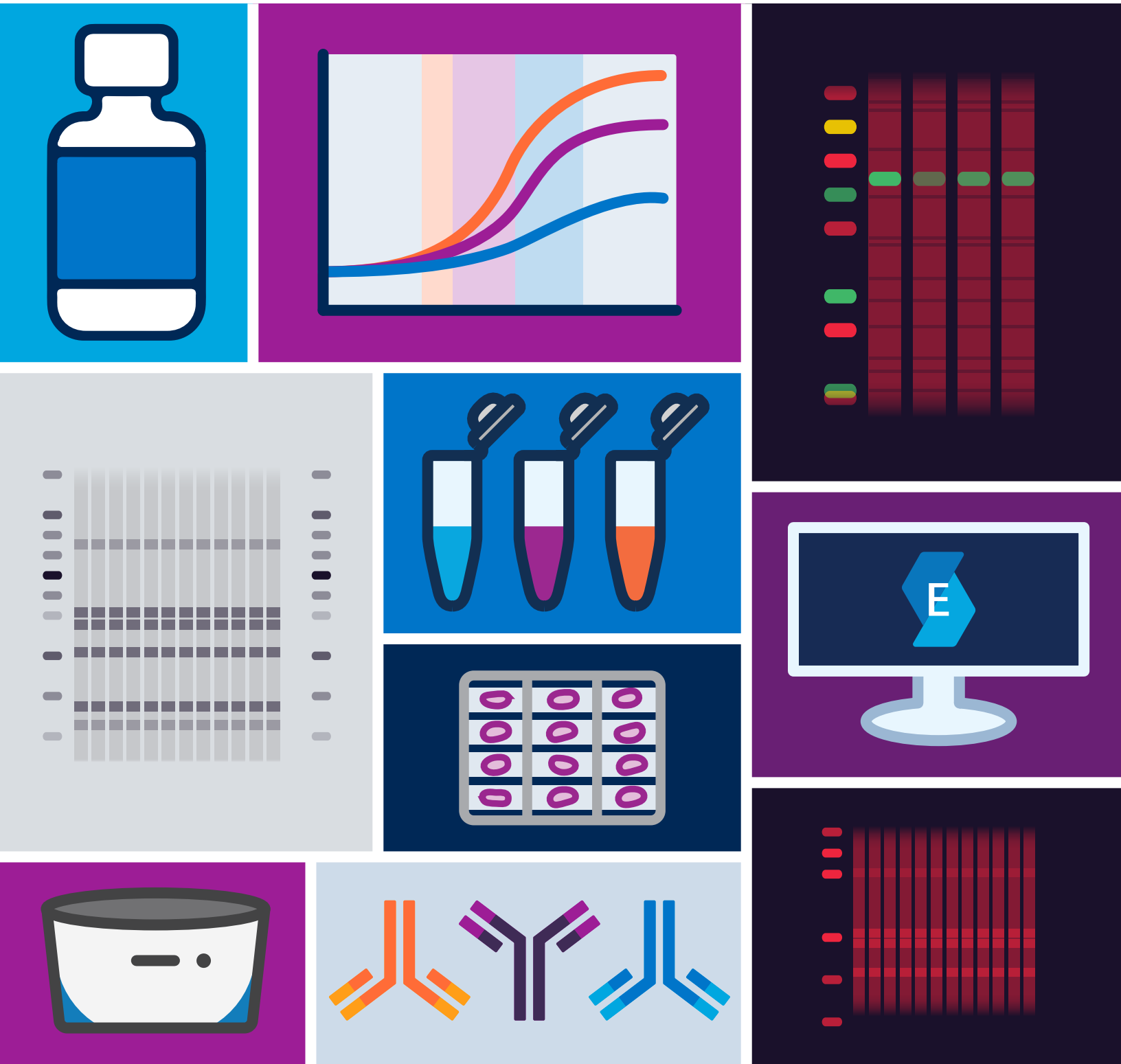
LI-COR®

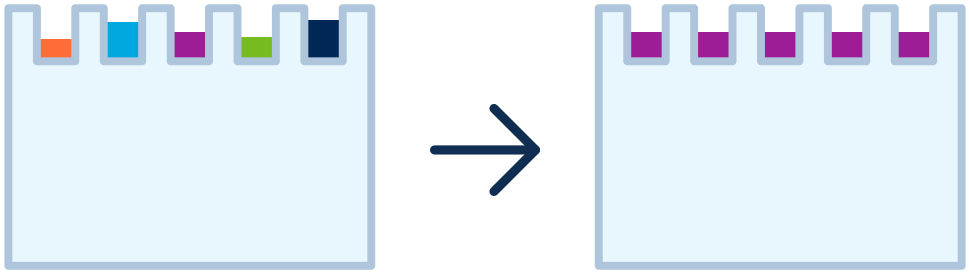
Normalization: Improving the Imperfect



What is normalization?

Normalization is a critical step in your workflow that corrects for small and unavoidable sample-to-sample and lane-to-lane variability.

In a perfect world, we would never need to normalize. In a perfect world, our pipettes would be expertly calibrated to load the exact amount of sample to every lane. However, no matter how hard we try, we cannot possibly account for all variability.



Normalization brings the imperfect a little closer to perfect and corrects for variability from sample loading, preparation, and transfer.

Normalization corrects for variability from:

Sample preparation

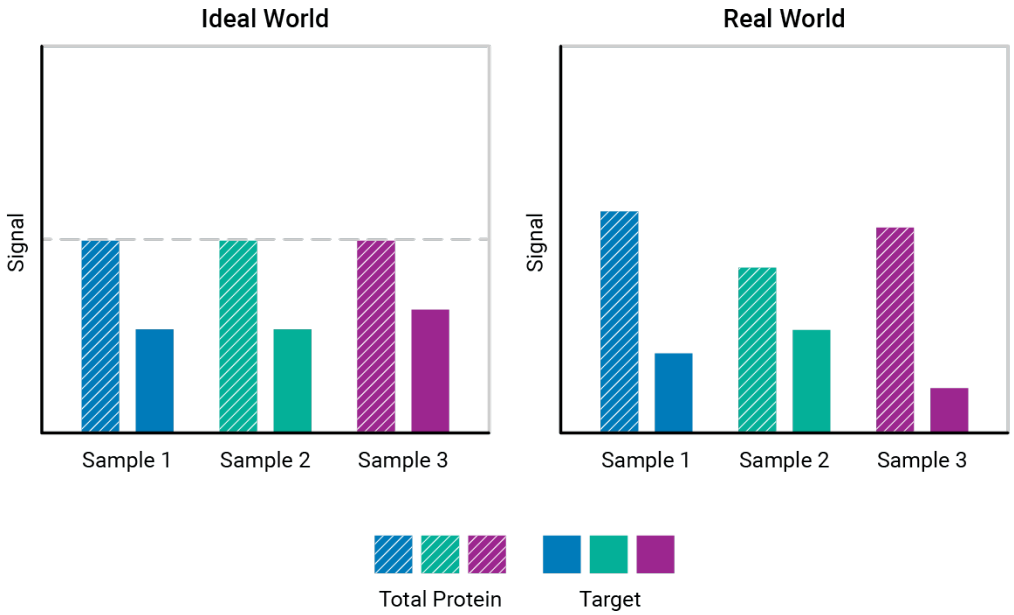
Loading

Transfer

Why is normalization important?

Normalization is important because the world is not perfect. Because we cannot account for every possible source of variability, we cannot be sure if the differences we see in our blots are caused by changes in expression or by variability.

Normalization helps minimize the effect variability has on your results.



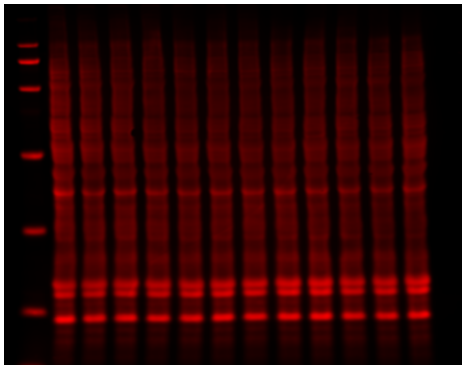
What makes a good internal loading control?

Normalization requires an internal loading control for comparison. An internal loading control is a reference protein or proteins used to indicate sample concentration. To be used as an internal loading control for normalization, this protein must be:

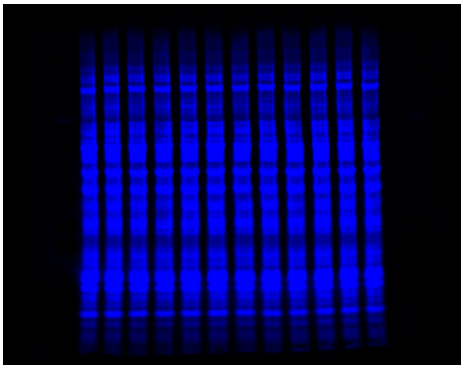
- Endogenous and present in all samples
- Unaffected by experimental conditions
- Detected in a linear range with target
- Compatible with target detection

What makes a good internal loading control?

Reagents play an important role in your chosen normalization strategy. Normalization to a housekeeping protein requires finding a primary antibody that is reliable and stably expressed. While total protein staining is preferred, find a strategy and reagents that perform well within your experimental design and imager capabilities.



Revert™ 700 Total Protein Stain



Revert 520 Total Protein Stain

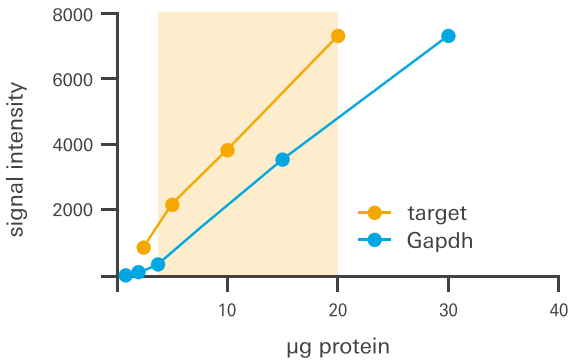
What are the main normalization strategies?

There are three main normalization strategies used in Western blotting:

- Housekeeping proteins
- Post-translational modification
- Total protein staining

Housekeeping protein

Housekeeping proteins (HKPs) are endogenous proteins, such as GAPDH, used as the loading control for normalization. While HKPs are found in all samples, they require an additional validation step. This is because they can be affected by experimental conditions and are vulnerable to biological variability. HKPs must also be revalidated any time experimental conditions change.

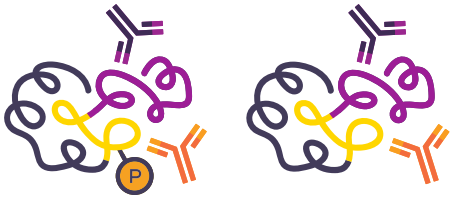


Things to remember:

- Validate a housekeeping protein before using it to normalize
- Both primary antibodies must be validated and revalidated if there is a change in conditions

Post-translational modification

This strategy uses the unmodified form of a target as a loading control when detecting a modified form of the target, such as phosphorylation. Using the target protein as the loading control has the advantage of being inherently unaffected by experimental conditions and eliminating error from HKP complications. However, it does require two primary antibodies, and both must be validated.



Things to remember:

- Requires two primary antibodies, one that recognizes the target and one that only recognizes the modified target
- Both primary antibodies must be validated

Total protein staining

Total protein staining uses a stain, such as Revert™ 700 Total Protein Stain, that stains all the proteins on a membrane. The target is then compared to the total amount of protein in each lane. Total protein staining is considered the normalization gold standard with many advantages.

Advantages of total protein staining

- Endogenous in all samples
- Inherently unaffected by experimental conditions
- Unaffected by biological variability
- Eliminates error from HKP complications, stripping, and reprobing
- Does not require separate validation

Things to remember:

- Considered the gold standard for normalization
- Recommended by publishers
- Minimizes variability and error

What normalization reagents does LI-COR offer?

LI-COR offers several primary antibodies to target HKPs for Western blot normalization. Our research and development team has thoroughly vetted these primary antibodies to ensure high quality and reliability.

LI-COR also offers two different total protein stains—Revert™ 700 Total Protein Stain and Revert 520 Total Protein Stain. Revert Total Protein Stains enable accurate and reliable normalization with a linear signal over a broad range making it easier to detect your target and Revert in the same linear range.

Revert Total Protein Stains:

- Stain in less than 10 minutes
- Work with your existing protocol
- Require no special equipment
- Are optimized for use on Odyssey® Imagers

In addition to normalization reagents, LI-COR offers many other reagents, including secondary antibodies and blocking buffers. LI-COR reagents have been optimized for use on Odyssey Imagers and ensure you get best-in-class support from experts familiar with the products your research relies on.

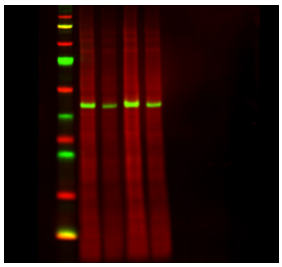
Looking to normalize your cell-based assays?

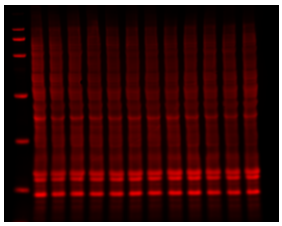
See our line of reagents for cell-based assays at **licor.com/celltag**

Revert™ 700 Total Protein Stain

Revert 700 Total Protein Stain is a near-infrared fluorescent stain that can be detected visibly or by using the 700 nm channel of an Odyssey® Imager. This stain does not covalently modify your sample, meaning it won't affect antibody binding or quantification.

Revert 700 provides high signal intensity with a broad linear range (1 - 60 µg of cell lysate) and can be used with multiplex detection to detect your target in the 800 nm channel and normalize using the 700 nm channel. Revert 700 can also be destained to enable you to detect targets in the 800 nm channel and 700 nm channel.

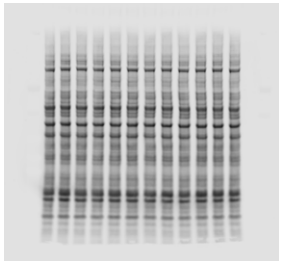


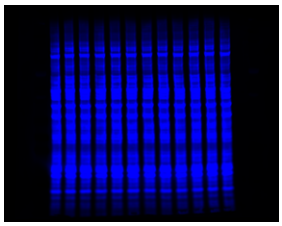


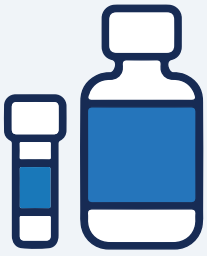
Revert 520 Total Protein Stain

Revert 520 Total Protein Stain is a visible fluorescent stain that can be detected visibly or by using the 520 nm channel. The only Odyssey Imager capable of detecting Revert 520 Stain is the Odyssey M Imager. Like Revert 700, Revert 520 Stain does not covalently modify your sample, meaning it won't affect antibody binding or quantification.

Revert 520 provides high signal intensity with a broad linear range and can be used with multiplex detection to detect targets in the 700 nm and 800 nm channels while normalizing to the 520 nm channel.

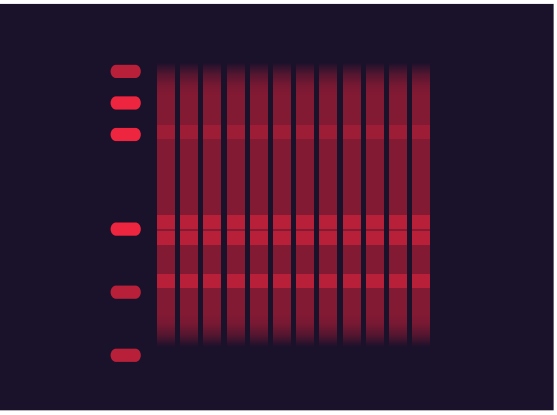


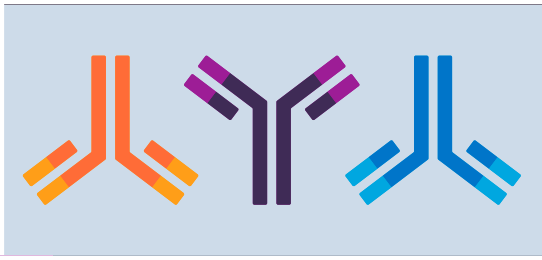
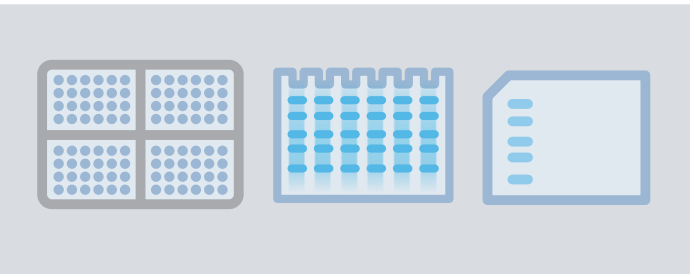
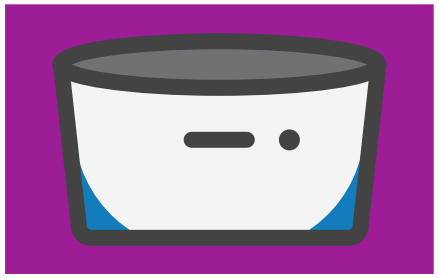
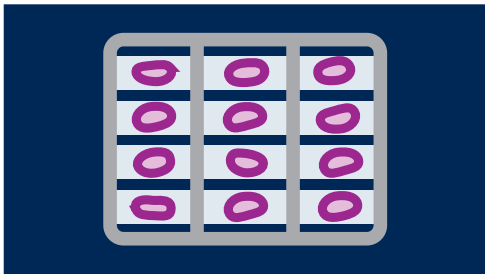




Find Revert Total Protein Stains and many other reagents at **licor.com/reagents**



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