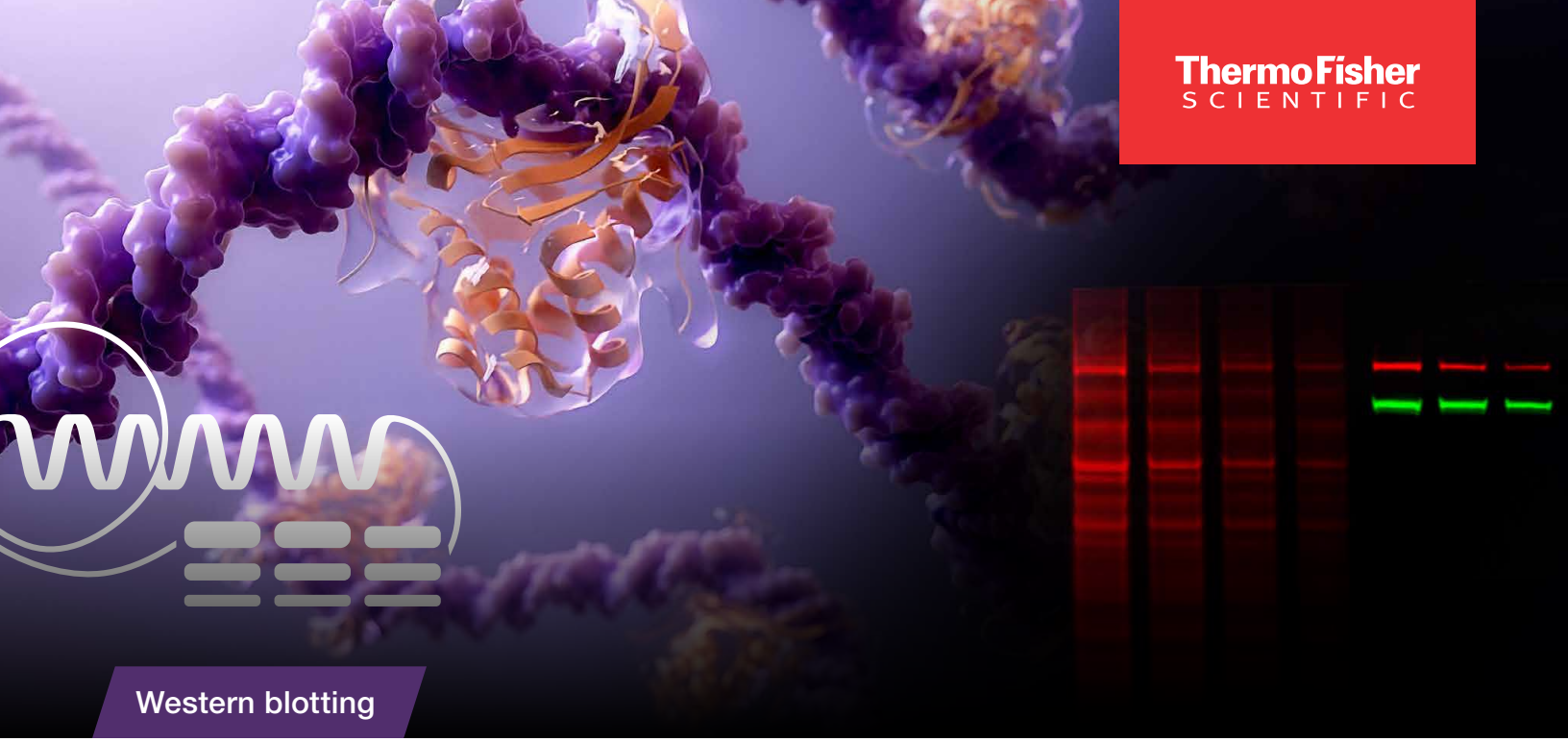


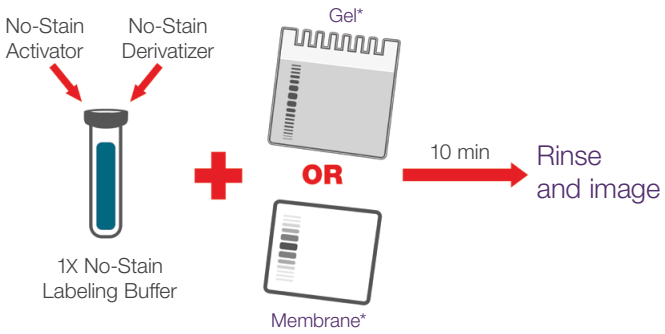
Western blotting

No-Stain Protein Labeling Reagent

Accurate, flexible total protein visualization and normalization of western blots and gels

Invitrogen™ No-Stain™ Protein Labeling Reagent is designed to help improve your western blot results with exceptional convenience.

- **Versatile**—use for fast total protein labeling of gels and membranes; compatible with all gel chemistries and with downstream gel transfer, western blotting, and mass spectrometry analysis
- **Fast protocol**—mix, incubate, and image; the reaction time is 10 minutes for gels and transferred membranes alike
- **Flexible visualization**—compatible with a wide range of excitation sources, including UV and green fluorescence light; optimal imaging conditions: excitation max. ~488 nm, emission max. 590 nm
- **Accurate total protein normalization**—imaging and total protein normalization analysis take only minutes with an Invitrogen™ iBright™ Imaging System
- **Broad linear dynamic range**—visualize 1–80 µg total protein loaded per well
- **Sensitive and stable signal**—protein bands are detected down to 20 ng and the signal is compatible with downstream antibody detection
- **Adjustable sensitivity**—increasing incubation time or doubling the reagent concentration produces a stronger signal to enable improved detection of low protein loads



* For Tris-glycine gels and membranes derived from Tris-glycine gel transfer, washing for 2 minutes with ultrapure water, 4 and 2 times, respectively, is necessary to remove glycine before performing labeling with No-Stain Protein Labeling Reagent.

Figure 1. A 10 min incubation with No-Stain Protein Labeling Reagent produces a strong, stable signal. Protein labeling can be performed on a gel or on a PVDF or nitrocellulose membrane after transfer. No-Stain reagent–labeled gels are compatible with downstream transfer and immunoprocessing.

invitrogen

Protein normalization using the No-Stain reagent vs. housekeeping proteins

Many leading journals have developed protein-normalization guidelines for submitting quantitative western blotting data, and housekeeping proteins are widely used for this purpose. However, the use of housekeeping proteins for protein normalization of western blots has its drawbacks, as the expression of housekeeping proteins can vary with experimental conditions and these proteins often have saturated western blotting signals.

Total protein normalization using the No-Stain Protein Labeling Reagent avoids the variability and inaccuracy of using housekeeping proteins, and eliminates the effort and cost of detecting them by immunoblotting, which may involve stripping and reprobing the blot.

In the experiment for which data are presented below, 10–50 µg loads of HeLa cell extract were separated on a gel and transferred to a Thermo Scientific™ Pierce™ Low-Fluorescence PVDF Transfer Membrane. The blot was labeled with No-Stain Protein Labeling Reagent and then immunoblotted with Invitrogen™ primary antibodies against β-actin (Cat. No. AM4302), GAPDH (Cat. No. 398600), and α-tubulin (Cat. No. 138000), followed by an Invitrogen™ goat anti-mouse IgG antibody conjugated to Invitrogen™ Alexa Fluor™ 680 dye (Cat. No. A21058). The graph in Figure 2 shows the linear signal response obtained using the No-Stain reagent for total protein normalization. Signals from the housekeeping proteins appear to plateau at higher loading levels. This is indicative of signal saturation, a condition in which the signal is no longer proportional to the relative amount of protein. This results in less accurate data normalization.

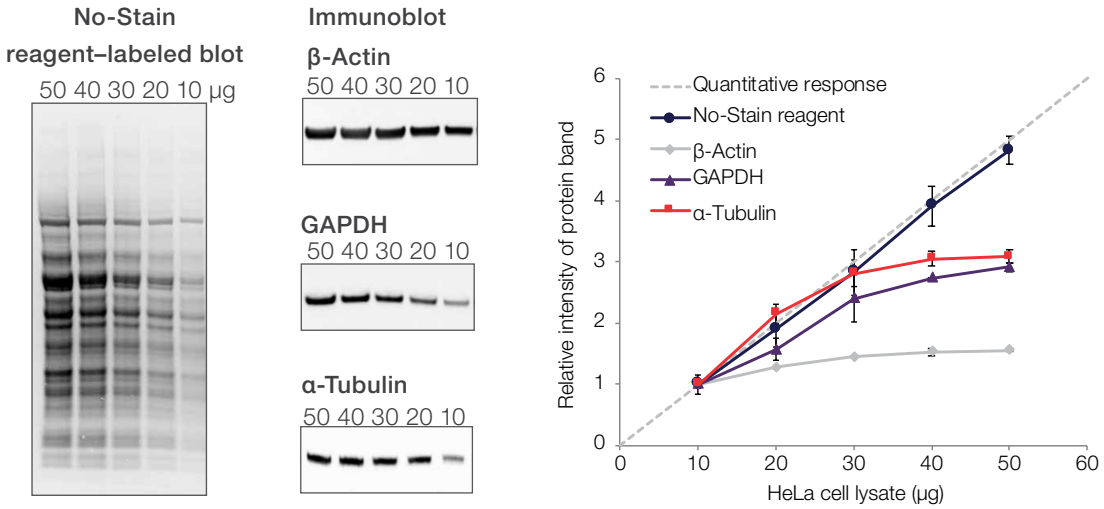


Figure 2. Total protein normalization with the No-Stain Protein Labeling Reagent. An Invitrogen™ Bolt™ 4–12% Bis-Tris Plus Gel was loaded with 10–50 µg of HeLa cell lysate and run with MES running buffer. Proteins from the gel were transferred to a low-fluorescence PVDF membrane using the Invitrogen™ Mini Blot Module and Bolt™ Transfer Buffer. The PVDF membrane was washed twice for 2 min with 20 mL of ultrapure water on a rotating platform, then labeled with 10 mL of No-Stain labeling reagent on a rotating platform for 10 min. The membrane was then washed three times for 2 min with 20 mL of ultrapure water on a rotating platform, followed by immunoblotting for β-actin, GAPDH, and α-tubulin. The blot was imaged using the Invitrogen™ iBright™ FL1500 Imaging System. The iBright system software was used to quantitate the total protein signal in the lanes. The linear regression value for the entire concentration range using the No-Stain reagent was determined (R^2 = 0.9990), whereas the R^2 for β-actin, GAPDH, and α-tubulin were 0.8851, 0.9438, and 0.8332, respectively.

Ordering information

Product	Qty	Cat. No.
No-Stain Protein Labeling Reagent	40 rxn	A44449
No-Stain Protein Labeling Reagent, trial size	10 rxn	A44717

Request a sample at **thermofisher.com/no-stain**