Determining the Appropriate Sample Load When Performing a Stain-Free Western Blot

Protocol

Abstract

Traditional western blot workflows require an appropriate sample load to detect both target proteins and loading control proteins. The discrepancy between these quantities often leads to oversaturated protein bands, forcing researchers to sometimes run duplicate gels or titrate down the antibody concentration in order to get quantitative results.

Stain-Free western blotting requires only a sample load appropriate for target protein detection. No reprobing of the blot for loading control proteins is needed. More importantly, the Stain-Free blots exhibit greater linearity in the load range of 10–50 µg of cell lysate.

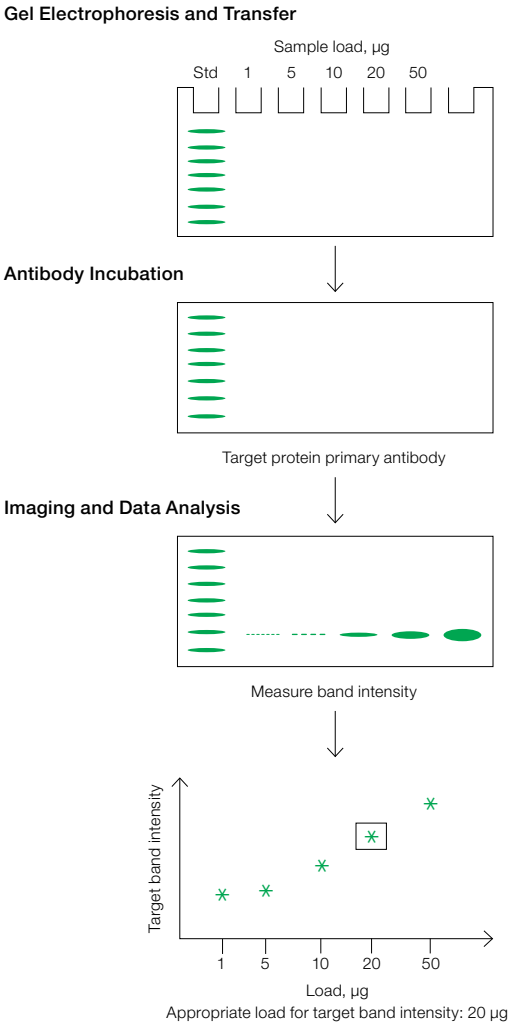
This protocol describes an assay development step to determine the right load for the detection of target proteins before the actual western blot experiment is carried out.

Protocol

1. Select a typical sample in which the target and loading control proteins are presented at an average level.
2. Load 1, 5, 10, 20, and 50 µg of protein sample to each lane.
3. After transfer, apply the target protein primary antibody to the blot.
4. Add substrate to develop the chemiluminescent signal and capture the signals using a CCD camera-based imager.
5. Use software to read the intensity of the target protein in each lane and plot those intensities against the protein load. Determine the linear dynamic range under the specific experimental conditions. Select the protein load that gives the quantitative reading in the linear dynamic range for the real experiment.

Note

- Procedure details are omitted for steps such as protein sample preparation, gel loading, gel electrophoresis, transfer, antibody incubation, etc. For details, please refer to the General Protocol for Western Blotting, bulletin 6376
- Once the experimental setup and conditions are established for the assay, do not change the sample load, transfer method, transfer time, antibody dilution, antibody incubation time, or temperature in subsequent experiments, as these factors may significantly change the detection signals



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