# Determining the Appropriate Sample Load for Western Blots

Bulletin 6362

Protocol

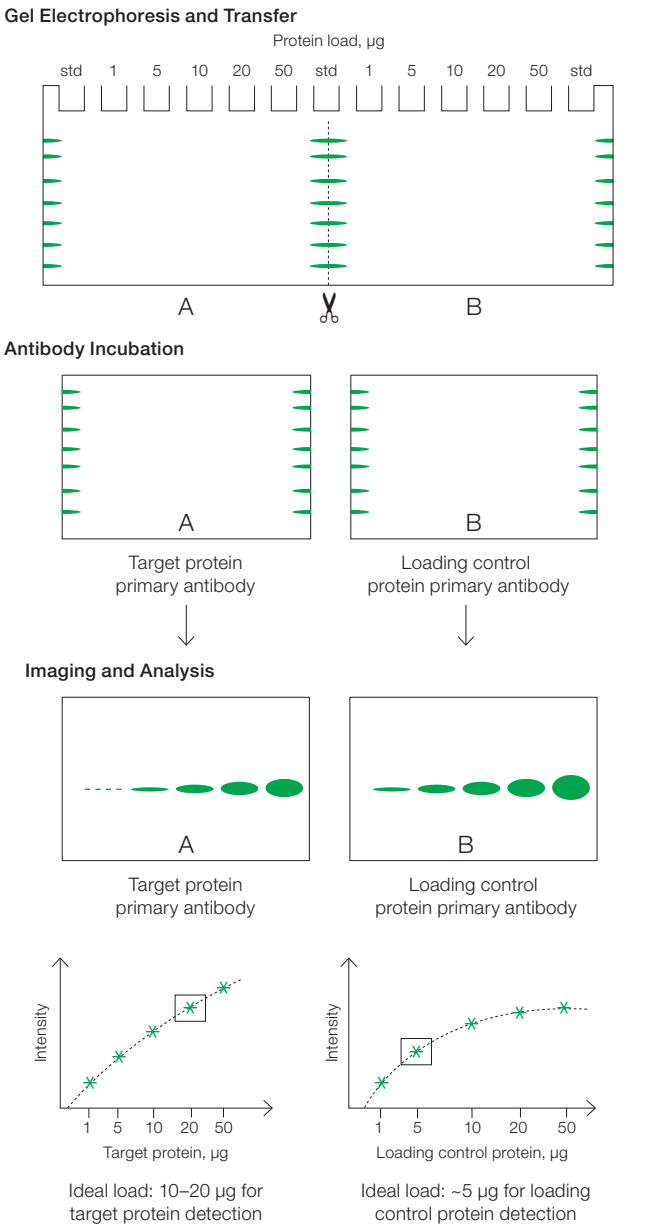
Reliable western blot data can be generated only when the proper sample amount of protein is used. Loading too much protein leads to signal saturation in western blots, yet too little produces weak signals. This protocol describes an assay development experiment to determine the appropriate protein load for target and control detection prior to performing the actual western blot experiment.

## Protocol:

1. Select a typical sample in which an average amount of target and loading control proteins are present.
2. Load 1, 5, 10, 20, and 50 µg of protein sample to two sets of lanes. Separate the two sets with a prestained protein standard.
3. After transfer, cut the membrane along the protein standards lane and split the membrane into 2 blots.
4. Apply the target protein primary antibody to one blot and the loading control protein antibody to the other.
5. Add substrate to develop the chemiluminescent signal and capture the signals using a CCD camera-based imager.
6. Using the software, read the target protein intensity in each lane and plot the intensity 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.
7. Repeat step 6 to determine the appropriate protein load for quantitative detection of the loading control protein.

**Note:**

- Procedure details are omitted for steps such as protein sample prep, gel load, 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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