Validating the Expression Consistency of a Housekeeping Protein

Bulletin 6366

Protocol

Housekeeping proteins (HKP) are typically used as loading controls because it is assumed that the HKP expression level remains consistent across samples. However, there is evidence that HKP expression levels change in many scenarios, including siRNA treatment, cell death, cell differentiation, etc. Therefore, validation of consistent HKP levels under specific experimental conditions is suggested prior to its selection as a loading control. This protocol describes how to validate the consistency of HKP expression before the actual western blot experiment is performed.

Protocol:

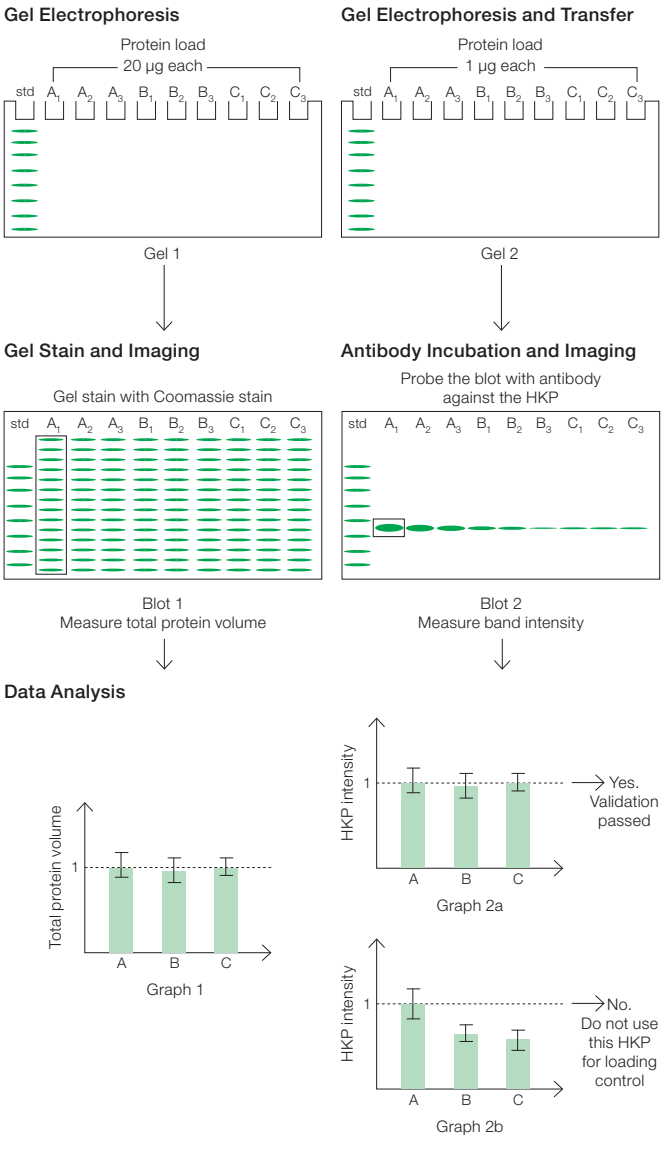
1. Collect at least three independent samples representative of each group (A, B, and C) that will be compared in a western blot experiment.
2. Prepare two identical gels for the experiment.
3. Load 20 µg cell lysate for each sample in Gel 1.
4. Load 1 µg cell lysate for each sample in Gel 2.

   Note: The small load is to ensure that immunodetection of the HKP is in the linear dynamic range.
5. Stain Gel 1 with Coomassie stain and acquire an image of the stained gel. Measure the total protein volume in each lane and plot the average for each group (A/B/C) against the group names in Graph 1. Normalize all data against the first group (A).

   Note: This graph will serve as a reference to see if the HKP levels are proportional to the total protein load.
6. Transfer proteins from Gel 2 to a membrane using a wet transfer or fast transfer system.
7. Following the antibody manufacturer instructions, apply the primary and secondary antibodies to detect the HKP on Blot 2.
8. Develop the blot with a substrate and use a CCD camera-based imager to capture the chemiluminescent signals. It is not recommended to use film.
9. Measure the HKP band intensity in each lane and plot the average intensity for each group against the group names in Graph 2. Normalize all data against the first group (A).
10. If the plots are consistent between graphs 1 and 2, the HKP expression is consistent among the different groups and this HKP can be used as a loading control (Graph 2a). Otherwise, do not use this particular HKP as a loading control (Graph 2b).

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



BIO-RAD