Determining the Appropriate Film Exposure Time

Protocol

Bulletin 6361

Film has a limited linear dynamic range for light detection and is often easily saturated by the chemiluminescent signals from the blot. This protocol describes a general procedure for determining the appropriate film exposure time.

Protocol

1. Select a substrate to develop the blot. It is recommended to use one with a very long signal duration, for example, Clarity™ western ECL substrate (cat #170-5060). A rapidly decaying chemiluminescent signal makes it extremely difficult to determine the appropriate film exposure time.
2. Expose film to the blot for 20 sec. Wait for it to develop.
3. If the protein bands on the 20 sec exposure appear too dark, reduce exposure increments to 2, 5, 10 and 60 sec. Develop the film.
4. If the protein bands on the 20 sec exposure appear too light, expose using increments of 10 sec, 1 min, 5 min, and 10 min. Develop the film.
5. Scan all film into images. Identify the most intense protein band on the film. Read the intensity of this protein band on all images and plot the band intensity against the exposure time.
6. Select the film where the signal of the most intense protein band is in the linear dynamic range to report the data.

Note:

- Procedure details are omitted for steps such us 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
- The exposure times used in this protocol may be varied depending on your experimental conditions