WB: Protocol - ECL Detection

In standard Western blot (WB) approaches, denatured protein samples are separated according to their molecular weight with SDS-PAGE (polyacrylamide gel electrophoresis) and transferred to a membrane. The analysis of different organs, cell-types, and subcellular fractions like membranes, versus cytosol or different organelles may also provide useful information about differential protein expression levels. Enhanced chemiluminescent (ECL) detection systems are very sensitive, but have a narrow linear detection range that can be used for protein quantification. In general, the experiment has to be carefully optimized for reliable results.

Important: Some proteins have special requirements for good separation (e.g. unboiled samples or special gel systems). Please refer to the **remarks** sections for western blotting on the respective data sheet.

Materials and reagents

- **Ponceau S staining solution:** 5% acetic acid, 0.1% Ponceau S
- **5% skimmed milk in Tris buffered saline with Tween 20 (5% skimmed milk-TBST):** 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5% (w/v) skimmed milk powder, 0.02% sodium azide, 0.1% Tween 20
- **Washing solution A:** 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20
- **Washing solution B:** 20 mM Tris-HCl, pH 7.5, 150 mM NaCl
- **Substrate solution:** Western Lightning® Plus-ECL PerkinElmer, Inc. or comparable product

Procedure

Separate the protein sample to be examined and a molecular weight standard using SDS-PAGE and transfer to a nitrocellulose membrane by electro-blotting. Follow the manufacturer's instructions for your SDS-PAGE and blotting device.

1. Stain the membrane with **Ponceau S staining solution** for several minutes at room temperature to check the efficiency of transfer.
2. Rinse the membrane in water to remove the Ponceau S staining solution and incubate in **5% skimmed milk-TBST** for 30 min on a lab shaker (gently rocking) at RT.
3. Incubate in fresh **5% skimmed milk-TBST** containing the primary antibody at the appropriate dilution and incubate for at least 2 h on a lab shaker at RT or overnight at 4°C.
4. Wash 3-4 times with **washing solution A** for 10 min each time.
5. Incubate in fresh **5% skimmed milk-TBST** containing the recommended secondary antibody (anti-mouse IgG, anti-rabbit IgG, resp.) at the appropriate dilution and incubate for at least 1 h on a lab shaker at RT.
6. Wash 3 times with **washing solution A** for 10 min each time.
7. Replace **washing solution A** with **washing solution B** and let equilibrate for 5 min.
8. Replace with fresh **substrate solution** and develop (X-ray film or ECL-reader). Exposure time can be shortened or extended, if signals are extremely strong or weak, resp.

Remarks

A very weak signal may be caused by the primary and/or secondary antibody concentration being too high. The ECL substrate solution has a limited capacity, and high amounts of local peroxidase can use up all the substrate within seconds before the picture is taken in your ECL reader.
Please try a lower concentration of primary and secondary antibodies in this case.

Note: *The SYSY standard protocol generates good results in the SYSY labs and may be used as a reference. However, to achieve the highest specific signal and lowest non-specific background signal, the best antibody concentration, incubation temperature, and incubation time must be determined individually.*