WB: Protocol - Fluorescent 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. Fluorescent detection systems have a broad linear detection range that can be used for accurate protein quantification. However, they are less sensitive compared to enhanced chemiluminescent (ECL) detection.

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
- **TBST:** 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20

Procedure for standard IgG based secondary antibodies

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 at RT.
3. Incubate in fresh **5% skimmed milk-TBST containing the primary antibody** at the appropriate dilution for at least 2 h on a lab shaker at RT or over-night at 4°C.
4. Wash 3-4 times with **5% skimmed milk-TBST** for 10 min each time.
5. Incubate with **TBST** containing the recommended fluorescent labeled secondary antibody (anti-mouse IgG, anti-rabbit IgG, resp.) at the appropriate dilution for at least 1 h on a lab shaker in the dark.
6. Wash 3 times **TBST** for 10 min each time.
7. Scan your membrane.

Procedure for FluoTag based secondary antibodies

NanoTag's unique FluoTags coupled to Licor dyes are ideal for time saving and quantitative western blotting with high precision.
Since Nanobodies are monovalent, they can be directly incubated with primary antibodies without forming non-functional clusters.

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 at RT.
3. During blocking, your membrane ad 1 µl of your primary antibody to 10 µl of your FluoTag-X2 solution and incubate for 30 min in the dark.
4. Dilute your antibody-FluoTag mix with **5% skimmed milk-TBST** and incubate your membrane for at least 2 h on a lab shaker in the dark.
5. Wash 3 times **TBST** for 10 min each time.
6. Scan your membrane.

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.*