Protocol for anti-Mouse IgG1 sdAb FluoTag (Cat. No. N2002-AF647-S)
Western Blot (WB)
Fluorescence 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 is a prerequisite for accurate protein quantification. However, they are less sensitive compared to enhanced chemiluminescent (ECL) detection.

Materials and reagents

- **Membrane staining solution (optional):** suitable staining solution (e.g. Ponceau S)
- **TBST - skimmed milk:** (20 mM Tris, 150 mM NaCl, 5% (w/v) skimmed milk powder, 0.1% Tween 20), pH 7.4
- **Washing solution A:** (20 mM Tris, 150 mM NaCl, 0.1% Tween 20), pH 7.4
- **Washing solution B:** (20 mM Tris, 150 mM NaCl), pH 7.4
- **Primary antibody:** mouse IgG of IgG1 isotype
- **Blotting membrane:** nitrocellulose or PVDF membrane. Low fluorescent background blotting-membranes are recommended.
- **Secondary antibody:** anti-Mouse IgG1 sdAb FluoTag Alexa 647-labeled

NanoTag's unique FluoTags coupled to fluorescent dyes are ideal for time saving and quantitative western blotting with high precision.
Since single domain antibodies (sdAbs) are monovalent, they can be directly incubated with purified primary antibodies without forming non-functional clusters. Unpurified formats like antiserum, culture supernatants and ascites are not recommended.

1. For Tissue or cell lysates prepare samples (10-20 µg / lane for a 10 lane mini-gel) in sample buffer according to the manufacturer instructions. If purified proteins or peptides are loaded use 1ng-1µg / lane for a 10 lane mini gel.

2. Separate the protein samples to be examined next to a molecular weight standard using SDS-PAGE and transfer to the blotting membrane by electro-blotting. Follow the manufacturer instructions for your SDS-PAGE and blotting device.

3. *Optional*: *Stain the membrane with membrane staining solution to check protein transfer.*

4. Incubate membrane in **5% TBST - skimmed milk** for 30 min on an orbital shaker at RT.

5. While blocking the membrane, pre-form complexes comprising primary antibodies and the secondary FluoTag reagent in a separate tube. For this, incubate 1 µg of your **primary antibody** with 4 µl of your FluoTag in 50 µl **TBST - skimmed milk** for 30 min in the dark.

6. Incubate the membrane in fresh **TBST - skimmed milk** containing the primary antibody FluoTag complex at the dilution recommended by the supplier of the primary antibody for at least 2 h on an orbital shaker at RT or overnight at 4°C.

7. Wash 3 times with **washing solution A** for 10 min each.

8. Replace **washing solution A** with **washing solution B** and equilibrate for 5 min.

9. Scan the membrane.

Note: This protocol has been validated in the NanoTag laboratories to ensure consistent and reliable staining results. However, for achieving the best specific signal with minimal background, the optimal antibody concentration, incubation temperature, and incubation duration should be optimized for each experiment.