



All protocols



Secondary nanobody labeling protocol

for easy and proper application of our labels

The combination of super bright and highly photostable *abberior* dyes and single-domain antibodies (sdAB, nanobodies) offers a fantastic solution for high-resolution imaging experiments where small labels, good penetration, and outstanding specificity are necessary.

Introduction

abberior offers a variety of excellent fluorescent dyes with properties optimized for labeling biomolecules, spectroscopic studies, and optical microscopy, particularly superresolution microscopy.

The combination of super bright and highly photostable *abberior* dyes and secondary nanobodies offers a fantastic solution for high-resolution imaging experiments where small labels, good penetration, and outstanding specificity are necessary.

Storage & reconstitution

FluoTag®-X2 monoclonal secondary nanobodies (Smart Secondaries®)

All fluorescent FluoTag®-X2 monoclonal secondary nanobodies from NanoTag Biotechnologies are lyophilized from PBS pH 7.4 with 2 % BSA (US-Origin) and shipped as lyophilized powder at ambient temperature. The lyophilized reagent can be stored at 2 - 8 °C for up to 12 months. Before usage, reconstitute and aliquot the reagent according to the detailed protocol below. After reconstitution in 500 µL, the final concentration is 10 µM dye and 5 µM nanobody.

- Prepare sterile 50% glycerol (v/v) in deionized water. If applicable, we recommend including 0.1% sodium azide as a preservative. Sodium azide should be avoided when staining live cells or conducting in vivo studies.

- Open the vial containing the lyophilized FluoTag® secondary reagent and add 500 µl of sterile 50 %glycerol (v/v) in deionized water.
- Mix gently and allow to sit at room temperature for approximately 5 min.
- **Optional:**Briefly spin down the vial for 2 min at 100 x g using a 50 ml conical tube with tissue paper at the bottom.
- Distribute into aliquots. Use small tubes and avoid aliquots below 20 µl.
- Storage: Working aliquot can be stored at -20°C for up to 4 weeks. Ideally store at -80°C (up to 6 months).

Polyclonal nanobodies

Polyclonal Nanobodies from Jackson ImmunoResearch are shipped in solution at ambient temperature. Upon arrival, the product can be stored at 4 °C for a short period of time. For long-term storage of up to one year add glycerol for a final concentration of 50 %, and store at -20 °C as a liquid.

Notes: Avoid repeated freeze-thaw cycles for all nanobody products. Therefore, it is recommended to split the *abberior* secondary nanobody solution into smaller aliquots. Minimize exposure to light to prevent photobleaching of the dye.

Indirect immunofluorescence staining with secondary nanobodies

The procedure has been successfully tested with our *abberior* dye conjugates for adherent cells grown on glass coverslips and has yielded consistent results in most instances, but may require further optimization for particular model organisms or experimental conditions.

Required reagents; not provided

- Phosphate-buffered saline pH 7.4 (PBS)
- Fixative depending on the primary antibody
- 0.1% – 0.5% Triton X-100 in PBS pH 7.4 (permeabilization buffer)
- 1% – 3% Bovine Serum Albumin + 0.1% Tween20 in PBS pH 7.4 (blocking buffer, PBT)
- Mounting Medium
- Glass coverslips with a glass thickness of $\sim 170 \mu\text{m}$ (No. 1.5 or No. 1.5H)

Note: We do not recommend using plastic coverslips because frequently only suboptimal imaging results are achieved. If possible, coverslips with grids, gratings, or similar should be avoided, as these structures can interfere with imaging causing aberrations that degrade image quality.

- Humid chamber
- Fluorescence microscope with suitable excitation light source and detection filter

Protocol for cultured cells

All steps are carried out at room temperature and in a petri dish unless stated otherwise.

1. Incubate cells with a fixative suitable for your primary antibody for 5 min to 30 min.

Note: When using Methanol as a fixative, step 2 can be skipped.

Optional: Quench with PBS supplemented with 0.1 M glycine or 0.1 M NH_4Cl for 10 min at RT.

2. Wash once with PBS.
3. Replace PBS with a permeabilization buffer and incubate the sample for 5 min to 15 min.
4. Wash cells 3 x 5 min in PBS.
5. Replace the PBS with a blocking buffer and incubate the sample for 30 min to 60 min.
6. Dilute the primary antibody to the concentration recommended by the supplier in the blocking buffer.

6. Take the coverslips out of the petri dish; remove the excess blocking buffer by placing the cover slip edge onto a piece of tissue paper. Transfer the coverslips into a humid chamber, cells facing upwards. Add the primary antibody onto the coverslips and incubate for 1 h in the humid chamber.
7. Remove excess primary antibodies by placing the cover slip edge onto a piece of tissue paper. Wash the cells in PBS (3 x 5 min) using a fresh petri dish.
8. Replace the PBS with a blocking buffer and incubate the sample for 30 min to 60 min.
9. Dilute the nanobody stock solution to the final staining concentration.

Note: For the *Fluotag® X2 monoclonal secondary nanobodies* in most applications, a dilution of 1:500 of a 5 μM stock solution is sufficient. However, staining protocols may vary with cell type and application. Dilution in PBS or PBT is possible.

Note: For the polyclonal nanobodies a dilution of 1:200 to 1:800 of a 1 mg/ml stock solution is sufficient. However, staining protocols may vary with cell type and application. To dilute the *polyclonal nanobodies*, BSA or other serum should be avoided, as the nanobodies can bind to free IgG antibodies within the serum and thus prevent optimal staining.

Optional: At this step *abberior* phalloidin can be added to the staining solution (1 Unit/ml).

10. Take the coverslips out of the petri dish. Remove excess blocking buffer by placing the cover slip edge onto a piece of tissue paper. Transfer the coverslips into a humid chamber, cells facing upwards. Add the secondary nanobody onto the coverslips and incubate for 1 h in the humid chamber under the exclusion of light.
11. Remove excess secondary nanobody by placing the cover slip edge onto a piece of tissue paper. Wash the cells in PBS (3 x 5 min) using a fresh petri dish.
12. Take the cover slip out of the washing solution, remove excess PBS by placing the cover slip edge onto a piece of tissue paper, and mount the coverslip with a suitable mounting medium.

Abbreviations

sdAB single-domain antibody
PBS Phosphate-buffered saline
PBT 1% – 3% Bovine Serum Albumin + 0.1% Tween20 in PBS
min Minute
h Hour