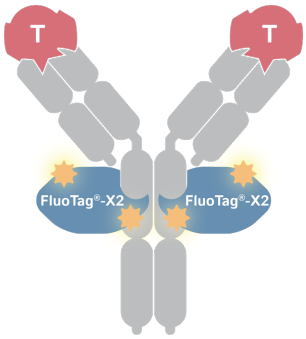
NanoTag Biotechnologies

Reconstitution and Storage

Fluorescent Smart Secondaries™

Smart Secondaries™ are NanoTag's secondary reagents derived from alpaca single-domain antibodies (sdAbs). They recognize conventional primary antibodies (immunoglobulins) with high species and isotype specificity. Fluorescently labeled **Smart Secondaries™** are a subgroup of NanoTag's **FluoTag®** reagents. In addition to conventional two-step immunofluorescence or Western blot applications, such fluorescent Smart Secondaries™ also allow for one-step immunolabeling and multiplexing with various primary antibodies of the same species in a single sample. All fluorescent Smart Secondaries™ are available as FluoTag®-X2 (see below). Additionally, NanoTag provides Smart Secondaries™ directed against rabbit IgGs in the FluoTag®-X4 format.

- **FluoTag®-X2:** Each sdAb molecule is coupled to two fluorophores. Due to the symmetry of the primary antibody, each primary antibody is accordingly labeled with up to four fluorophores (see Figure).
- **FluoTag®-X4**: Enhanced variant combining two distinct FluoTag®-X2 molecules, each binding to a different eptitope on a single primary antibody. The FluoTag®-X4 format increase the visibility of the target (T) by bringing up to eight fluorophores to each primary antibody, making it ideal for generating bright images.



All fluorescent Smart Secondaries™ 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 as follows:

- FluoTag®-X2: 10 µM dye, 5 µM sdAb
- FluoTag®-X4: 10 µM dye, 2.5 µM of each sdAb

Protocol: Reconstitution of Fluorescent Smart Secondaries™

1. 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.
2. Open the vial containing the lyophilized Smart Secondary™ reagent.
3. Add 500 µL of sterile 50% glycerol (v/v) in deionized water.
4. Mix gently and allow to sit at room temperature for ~5 min.
5. 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.
6. Distribute into aliquots. Use small tubes and avoid aliquots below 20 µL.
7. Storage:
   - Short-term: Working aliquot can be stored at -20°C for up to 4 weeks.
   - Long-term: Ideally store at -80°C (up to 6 months).

Notes:
- Avoid repeated freeze-thaw cycles.
- Minimize exposure to light to prevent photobleaching of the dye.

Only for research applications, not for diagnostic or therapeutic use!

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