SYSY Antibodies

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RFP sdAb

Cat.No. N0404-AF750-L; Single Domain camelid antibody, 200 µl FluoTag-X4

Data Sheet

Reconstitution/ Storage	200 µl purified antibody, lyophilized from PBS, fluorescence-labeled with Alexa 750. Albumin was added for stabilization. For reconstitution refer to the NanoTag reconstitution and storage instructions. Reconstitute immediately upon receipt! Avoid bright light when working with the antibody to minimize photo bleeching of the fluorescent dye. For detailed information, see back of the data sheet.
Applications	**ICC**: 1 : 500 **FACS**: yes
Label	Alexa 750, two fluorophores coupled to two FluoTags each
Clone	2B12/2A1
Immunogen	Recombinant protein corresponding to AA 1 to 225 from sea anemone RFP (UniProt Id: Q9U6Y8)
Specificity	mRFP and derivatives like mCherry, mScarlet-i, tdTomato, dsRed, mOrange

TO BE USED IN VITRO / FOR RESEARCH ONLY
NOT TOXIC, NOT HAZARDOUS, NOT INFECTIOUS, NOT CONTAGIOUS

Background

Red fluorescent protein RFP is a common fusion protein used in cell biology to follow the localization and expression of proteins of interest. RFP is known for its rapid maturation and high resistance to photobleaching and therefore well suited for multi color imaging.

Unlabeled variants and several modifications of sdAbs like biotin, fluorophore or DBCO conjugation are available.

In **FluoTag®-Q** each fluorophore is coupled to exactly one FluoTag, which in turn binds to its target molecule in a monovalent fashion. The high binding affinity and a coupling efficiency of > 95% guarantees a highly linear relation between the number of target molecules and the intensity of fluorescence. This enables a direct count of the target molecule of interest. The fluorophore is located exceptionally close to the recognized epitope (< 1.5 nm), which is ideal for all microscopy techniques.

In **FluoTag®-X** two fluorophore molecules are site-specifically coupled to each FluoTag molecule. Therefore, the reagent simultaneously targets up to four fluorophores (in X4 variants) to the protein of interest, which ensures extra-bright signals. Owing to the small size of the FluoTags, the distance between the target epitope and each fluorophore is ~ 3 nm.
In comparison to detection systems using conventional antibodies, FluoTag-X can thus improve the localization accuracy by 10-15 nm. Both features - superior brightness and precise fluorophore placement - render the FluoTag-X products excellent tools for all microscopy techniques.

Selected General References

Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein.
Shaner NC et al. Nat. Biotechnol. (2004) PubMed:15558047

Access the online factsheet including applicable protocols at https://sysy.com/product/N0404-AF750-L or scan the QR-code.

FAQ - How should I store my antibody?

Shipping Conditions

- All SYSY antibodies and control proteins/peptides are shipped lyophilized (vacuum freeze-dried). In this form, they remain stable without loss of quality at ambient temperatures for several weeks.

Storage of Sealed Vials after Delivery

- **Unlabeled** and **biotin-labeled antibodies** and **control proteins** should be stored at **4°C** before reconstitution. **Do not freeze lyophilized antibodies**. Temperatures below 0°C may impair performance.
- **Fluorescence-labeled antibodies** should be reconstituted immediately upon receipt. Long-term storage of lyophilized fluorophore-conjugates may cause aggregation.
- **Control peptides** should be stored at -20°C before reconstitution.

Long Term Storage after Reconstitution (General Considerations)

- **Do not use frost-free ("no-frost") freezers.** These units periodically warm to remove ice buildup, causing freeze–thaw cycles that can damage antibodies.
- Store vials in areas with minimal temperature fluctuation - preferably toward the back of the freezer, not on the door.
- Aliquot reconstituted antibodies and store at –20°C to –80°C.
- Avoid very small aliquots (<20 µL), as evaporation and adsorption to tube surfaces can reduce antibody concentration and activity.
- Use the smallest practical storage vial to minimize surface area.
- Adding glycerol to a final concentration of 50% prevents freezing at -20°C, allowing storage in liquid form and effectively avoiding freeze–thaw cycles.

Product Specific Hints for Storage

Control proteins / peptides

- Store at -20°C to -80°C

Monoclonal Antibodies

- **Ascites and hybridoma supernatant:** Store at -20°C to -80°C
  Prolonged storage at 4°C is not recommended, as proteases present in ascites may degrade antibodies.
- **Purified IgG:** Store at -20°C to -80°C
  Adding a carrier protein (e.g., BSA) enhances long-term stability.
  Many SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

Polyclonal Antibodies

- **Crude antisera:** Can be stored at 4°C with antimicrobials added, but -20°C to -80°C is preferred
- **Affinity-purified antibodies:** Less stable than antisera; store at -20°C to -80°C
  Adding a carrier protein such as BSA improves long-term stability.
  Most SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

Fluorescence-labeled Antibodies

- Store as a liquid with 1:1 (v/v) glycerol at -20°C, and protect from light exposure

Avoid repeated freeze-thaw cycles for all antibodies!

FAQ - How should I reconstitute my antibody?

Reconstitution

- All purified SYSY antibodies are lyophilized from PBS. To reconstitute the antibody in PBS, add the volume of deionized water specified in the corresponding datasheet. If a larger final volume is desired, first add the recommended amount of water, then adjust with PBS and, if needed, add a stabilizing carrier protein (e.g., BSA) to a final concentration of 2%. Some SYSY antibodies already contain albumin; please take this into account before adding additional carrier protein.

For complete reconstitution, carefully remove the vial cap. After adding water, briefly vortex the solution. To collect the liquid at the bottom of the vial, place the vial inside a 50 ml centrifuge tube padded with paper and centrifuge briefly.

- If desired, small amounts of azide or thimerosal may be added to prevent microbial growth. This is particularly recommended when storing an aliquot at 4°C.
- After reconstitution of fluorescence-labeled antibodies, add glycerol 1:1 (v/v) to achieve a final concentration of 50%. This prevents freezing at –20°C and keeps the antibody in liquid form, effectively avoiding freeze–thaw cycles.
- Glycerol may also be added to unlabeled primary antibodies as a general measure to prevent freeze–thaw damage.
- For further guidance, please refer to our **storage tips** and recommendations for reconstituted antibodies, control peptides, and control proteins.