SYSY Antibodies

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RFP sdAb

Cat.No. N0404-At488-L; Single Domain camelid antibody, 200 µl FluoTag-X4

Data Sheet

Reconstitution/ Storage	200 µl purified antibody, lyophilized from PBS, fluorescence-labeled with ATTO® 488. Albumin was added for stabilization. For **reconstitution** add 200 µl H_2O. Either add 1:1 (v/v) glycerol, then aliquot and store at -20°C until use, or store aliquots at -80°C without additives. 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.
Storage	Up to three months: -20°C Up to 12 months: -80°C or below Protect form light!
Applications	**WB**: not recommended **IP**: N/A **ICC**: 1 : 250 **IHC**: not tested yet **IHC_P**: not tested yet
Label	ATTO 488, two fluorophores coupled to two FluoTags each
Clone	2B12-2A1
Subtype	single domain
Immunogen	Recombinant protein corresponding to AA 1 to 225 from sea anemone RFP (UniProt Id: Q9U6Y8)
Specificity	Recognizes most common red fluorescent proteins like mRFP, mCherry, dsRed 1/2 and tdTomato. Does not cross-react with GFP or mTagBFP derivatives.

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, Campbell RE, Steinbach PA, Giepmans BN, Palmer AE, Tsien RY
Nature biotechnology (2004) 2212: 1567-72. .

Access the online factsheet including applicable protocols at https://sysy.com/product/N0404-At488-L or scan the QR-code.

FAQ - How should I store my antibody?

Shipping Conditions

- All our antibodies and control proteins / peptides are shipped lyophilized (vacuum freeze-dried) and are stable in this form 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. **They must not be stored in the freezer when still lyophilized!** Temperatures below zero may cause loss of performance.
- **Fluorescence-labeled antibodies** should be reconstituted immediately upon receipt. Long term storage (several months) may lead to aggregation.
- **Control peptides** should be kept at -20°C before reconstitution.

Long Term Storage after Reconstitution (General Considerations)

- The storage freezer must not be of the frost-free variety ("no-frost freezer"). This cycle between freezing and thawing (to reduce frost-build-up), which is exactly what should be avoided. For the same reason, antibody vials should be placed in an area of the freezer that has minimal temperature fluctuations, for instance towards the back rather than on a door shelf.
- Aliquot the antibody and store frozen (-20°C to -80°C). Avoid very small aliquots (below 20 µl) and use the smallest storage vial or tube possible. The smaller the aliquot, the more the stock concentration is affected by evaporation and adsorption of the antibody to the surface of the storage vial or tube. Adsorption of the antibody to the surface leads to a substantial loss of activity.
- The addition of glycerol to a final concentration of 50% lowers the freezing point of your stock and keeps your antibody at -20°C in liquid state. This efficiently avoids freeze and thaw cycles.

Product Specific Hints for Storage

Control proteins / peptides

- Store at -20°C to -80°C.

Monoclonal Antibodies

- **Ascites** and **hybridoma supernatant** should be stored at -20°C up to -80°C. **Prolonged storage at 4°C is not recommended!** Unlike serum, ascites may contain proteases that will degrade the antibodies.
- **Purified IgG** should be stored at -20°C up to -80°C. Adding a carrier protein like BSA will increase long term stability. Many of our antibodies already contain carrier proteins. Please refer to the data-sheet for detailed information.

Polyclonal Antibodies

- **Crude antisera**: With anti-microbials added, they may be stored at 4°C. However, frozen storage (-20°C up to -80°C) is preferable.
- **Affinity purified antibodies**: Less robust than antisera. Storage at -20°C up to -80°C is recommended. Adding a carrier protein like BSA will increase long term stability. Most of our antibodies already contain carrier proteins. Please refer to the data-sheet for detailed information.

Fluorescence-labeled Antibodies

- Store as a liquid with 1 : 1 (v/v) glycerol at -20°C. Protect these antibodies from light exposure.

Avoid repeated freeze-thaw cycles for all antibodies!

FAQ - How should I reconstitute my antibody?

Reconstitution

- All our purified antibodies are lyophilized from PBS. To reconstitute the antibody in PBS, add the amount of deionized water given in the respective datasheet. If higher volumes are preferred, add water as mentioned above and then the desired amount of PBS and a stabilizing carrier protein (e.g. BSA) to a final concentration of 2%. Some of our antibodies already contain albumin. Take this into account when adding more carrier protein.
  For complete reconstitution, carefully remove the lid. After adding water, briefly vortex the solution. You can spin down the liquid by placing the vial into a 50 ml centrifugation tube filled with paper.
- If desired, add small amounts of azide or thimerosal to prevent microbial growth. This is especially recommended if you want to keep an aliquot a 4°C.
- After reconstitution of fluorescence-labeled antibodies, add 1 : 1 (v/v) glycerol to a final concentration of 50%. This lowers the freezing point of your stock and keeps your antibody in liquid state at -20°C.
- Glycerol may also be added to unlabeled primary antibodies. It is a suitable way to avoid freeze-thaw cycles.
- Please refer to our **tips and hints for subsequent storage** of reconstituted antibodies and control peptides and proteins.