SYSY Antibodies

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mCLING

Cat.No. 710 006AT647N; , 5 nmol mCling

Data Sheet

Reconstitution/ Storage	5nmol mCLING labeled with ATTO® 647N in 100 µl PBS (lyophilized). For reconstitution add 100 µl H_2O, then aliquot and store at -80°C until use. Reconstitute immediately upon receipt! Avoid bright light when working with the probe to minimize photo bleeching of the fluorescent dye. For detailed information, see back of the data sheet.
Applications	**ICC**: 1 : 50 up to 1 : 250 (1 - 0.2 nmol/ml) **IHC**: 1 : 25 up to 1 : 50 (2 - 1 nmol/ml)
Label	ATTO 647N
Remarks	Due to the positive charge of mCLING, negatively charged coatings of cover-slips should be avoided. We recommend a positively charged coating like poly-L-lysine (PLL). mCLING is a fixable dye but paraformaldehyde alone is not able to fix this molecule sufficiently. Therefore, a mixture of 4 %paraformaldehyde (PFA) and 0.2 % glutaraldehyde is strongly advised. For detailed protocols see Revelo NH & Rizzoli SO, 2016.

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

Background

The **m**embrane-binding fluorophore-**c**ysteine-**l**ys**in**e-palmtoyl **g**roup (**mCLING**) is a new probe that selectively binds to the plasma membrane. It is taken up during endocytosis and, in contrast to conventional membrane dyes, remains attached to membranes after fixation and permeabilization and can therefore be combined with immunostaining and super-resolution microscopy.
mCLING was used so far in mammalian-cultured cells, yeast, bacteria, primary cultured neurons, Drosophila melanogaster larval neuromuscular junctions, and mammalian tissue.

Selected References for 710 006AT647N

A new probe for super-resolution imaging of membranes elucidates trafficking pathways.
Revelo NH, Kamin D, Truckenbrodt S, Wong AB, Reuter-Jessen K, Reisinger E, Moser T, Rizzoli SO
The Journal of cell biology (2014) 2054: 591-606. . **ICC, IHC**

The Membrane Marker mCLING Reveals the Molecular Composition of Trafficking Organelles.
Revelo NH, Rizzoli SO
Current protocols in neuroscience (2016) 74: 2.25.1-21. . **ICC, IHC**

One-step nanoscale expansion microscopy reveals individual protein shapes.
Shaib AH, Chouaib AA, Chowdhury R, Altendorf J, Mihaylov D, Zhang C, Krah D, Imani V, Spencer RKW, Georgiev SV, Mougios N, et al.
Nature biotechnology (2024) : . . **EXM; tested species: rat**

Endophilin-A coordinates priming and fusion of neurosecretory vesicles via intersectin.
Gowrisankaran S, Houy S, Del Castillo JGP, Steubler V, Gelker M, Kroll J, Pinheiro PS, Schwitters D, Halbsgut N, Pechstein A, van Weering JRT, et al.
Nature communications (2020) 111: 1266. . **UPTAKE; tested species: mouse**

Oligodendroglial macroautophagy is essential for myelin sheath turnover to prevent neurodegeneration and death.
Aber ER, Griffey CJ, Davies T, Li AM, Yang YJ, Croce KR, Goldman JE, Grutzendler J, Canman JC, Yamamoto A
Cell reports (2022) 413: 111480. . **UPTAKE; tested species: mouse**

Vivaxin genes encode highly immunogenic, non-variant antigens on the Trypanosoma vivax cell-surface.
Romero-Ramirez A, Casas-Sánchez A, Autheman D, Duffy CW, Brandt C, Clare S, Harcourt K, André MR, de Almeida Castilho Neto KJG, Teixeira MMG, Machado RZ, et al.
PLoS neglected tropical diseases (2022) 169: e0010791. . **ICC**

Visualizing cellular and tissue ultrastructure using Ten-fold Robust Expansion Microscopy (TREx).
Damstra HGJ, Mohar B, Eddison M, Akhmanova A, Kapitein LC, Tillberg PW
eLife (2022) 11: . . **ICC; tested species: human**

CtBP1-Mediated Membrane Fission Contributes to Effective Recycling of Synaptic Vesicles.
Ivanova D, Imig C, Camacho M, Reinhold A, Guhathakurta D, Montenegro-Venegas C, Cousin MA, Gundelfinger ED, Rosenmund C, Cooper B, Fejtova A, et al.
Cell reports (2020) 307: 2444-2459.e7. . **ICC; tested species: mouse**

Opposite Surfaces of the Cdc15 F-BAR Domain Create a Membrane Platform That Coordinates Cytoskeletal and Signaling Components for Cytokinesis.
Snider CE, Chandra M, McDonald NA, Willet AH, Collier SE, Ohi MD, Jackson LP, Gould KL
Cell reports (2020) 3312: 108526. . **ICC; tested species: fission yeast**

Integrative characterization of the near-minimal bacterium Mesoplasma florum.
Matteau D, Lachance JC, Grenier F, Gauthier S, Daubenspeck JM, Dybvig K, Garneau D, Knight TF, Jacques PÉ, Rodrigue S
Molecular systems biology (2020) 1612: e9844. . **ICC**

A versatile and customizable low-cost 3D-printed open standard for microscopic imaging.
Diederich B, Lachmann R, Carlstedt S, Marsikova B, Wang H, Uwurukundo X, Mosig AS, Heintzmann R
Nature communications (2020) 111: 5979. . **ICC; tested species: e. coli**

Access the online factsheet including applicable protocols at https://sysy.com/product/710006AT647N 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.