SYSY Antibodies

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mCLING-ATTO 488

Cat.No. 710 006AT3; , 5 nmol mCling

Data Sheet

Reconstitution/ Storage	5nmol mCLING labeled with ATTO® 488 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 488
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 006AT3

A fixable probe for visualizing flagella and plasma membranes of the African trypanosome.
Wiedeman J, Mensa-Wilmot K
PloS one (2018) 135: e0197541. . **ICC, FACS**

Otoferlin Depletion Results in Abnormal Synaptic Ribbons and Altered Intracellular Calcium Levels in Zebrafish.
Manchanda A, Chatterjee P, Bonventre JA, Haggard DE, Kindt KS, Tanguay RL, Johnson CP
Scientific reports (2019) 91: 14273. . **UPTAKE; tested species: zebrafish**

HIV-1 Capsid Uncoating Is a Multistep Process That Proceeds through Defect Formation Followed by Disassembly of the Capsid Lattice.
Gifford LB, Melikyan GB
ACS nano (2024) 184: 2928-2947. . **ICC; tested species: human**

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**

Truncation of the otoferlin transmembrane domain alters the development of hair cells and reduces membrane docking.
Manchanda A, Bonventre JA, Bugel SM, Chatterjee P, Tanguay R, Johnson CP
Molecular biology of the cell (2021) : mbcE20100657. . **UPTAKE; tested species: zebrafish**

Preformed Ω-profile closure and kiss-and-run mediate endocytosis and diverse endocytic modes in neuroendocrine chromaffin cells.
Shin W, Wei L, Arpino G, Ge L, Guo X, Chan CY, Hamid E, Shupliakov O, Bleck CKE, Wu LG
Neuron (2021) 10919: 3119-3134.e5. . **UPTAKE; tested species: cow**

Visualization of Membrane Pore in Live Cells Reveals a Dynamic-Pore Theory Governing Fusion and Endocytosis.
Shin W, Ge L, Arpino G, Villarreal SA, Hamid E, Liu H, Zhao WD, Wen PJ, Chiang HC, Wu LG
Cell (2018) : . . **ICC**

Selected General References

Nanoscale architecture of the Schizosaccharomyces pombe contractile ring.
McDonald NA, Lind AL, Smith SE, Li R, Gould KL
eLife (2017) 6: . .

SWAP70 Organizes the Actin Cytoskeleton and Is Essential for Phagocytosis.
Baranov MV, Revelo NH, Dingjan I, Maraspini R, Ter Beest M, Honigmann A, van den Bogaart G
Cell reports (2016) 176: 1518-1531. .

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. .

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. .

Access the online factsheet including applicable protocols at https://sysy.com/product/710006AT3 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.