

SySy mCLING Labeling Kit

Cat. No. 710-MCK

Intended Use

The SYSY mCLING labeling kit allows the selective labeling with any maleimid activated fluorophore.

Background

The membrane-binding fluorophore-cysteine-lysine-palmitoyl group (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.

Kit content

710-MCK A: unlabeled mCling, 50nmol (lyophilized)
710-MCK B: mCLING purification column
710-MCK C: Washing buffer, 10ml
710-MCK D: Stop solution, 0.5ml
710-MCK E: Elution buffer, 1ml
710-MCK F: Neutralization buffer, 0.5ml

Storage and stability

All components of the kit are stable at 2-8°C. Shelf life before use: 6 months.

Procedural notes

- Dissolve mCLING with 100µl PBS just before starting and transfer to a 1.5ml Eppendorf tube. Add 100-200nmol of maleimid activated dye of choice. The dye should be dissolved in DMSO at a concentration of 10mg/ml.
- Incubate for 2h at room temperature rotating head over tail.
- Add 10µl stop solution and incubate for 20min at room temperature.
- Remove caps of the purification column and place into a 1.5 ml Eppendorf tube and let drop out storage buffer completely by gravity flow.
- Transfer column to a fresh 2ml Eppendorf tube and apply 500µl washing buffer and let drop out completely by gravity flow.
- Transfer column to a fresh Eppendorf tube and apply labeling reaction to the column and collect the flow through.
- Transfer column to a fresh 2ml Eppendorf tube and apply 500µl washing buffer.
- Transfer column to a fresh 1.5ml Eppendorf tube, apply 200µl elution buffer and collect eluate. If you use a visible dye, you can also try to collect only the coloured eluate to get a more concentrated sample.
- Add 20µl (or 1/10 v/v) of neutralization buffer and mix well.
- Store labeled mCLING at -80°C.

Calculation of the amount of labeled mCLING

Dilute the eluate 1:50 with PBS or water and measure at wavelength appropriate for the dye used in the labeling reaction.

$$X \text{ nmol}/100\mu\text{l} = \text{OD}_{\text{y nm}} * 5 * 10^6 / \text{extinction coefficient}_{\text{dye}}$$

Precautions for handling

Do not use expired reagents.

This kit is for research purpose only. Do not use for clinical diagnosis.

Manufacturer

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