Chromeo™ LT Live Cell Membrane Staining Kit

(version A1)

Catalog No. 15007

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Introduction

Cell membranes are selective lipid barriers that make possible compartmentation, control of the influx and efflux of distinct molecules as well as communication with the cell's surroundings. Moreover, intracellular lipid bilayers create cell organelles like mitochondria, lysosomes, endoplasmic reticulum and the nucleus that enable the cell to perform a variety of diverse metabolic functions at the same time through the establishment of the different environments produced by this compartmentation.

Fluorescent stains that specifically intercalate into membranes can be used not only to label the different cellular compartments, but also for live cell imaging. The Chromeo™ LT Live Cell Membrane Stain* allows lipid structure labeling after a short incubation time of 10 minutes at 37°C without extensive washing steps. The stain can be excited from 400 nm to 490 nm, and is compatible with the 488 nm argon laser line. The complex emits bright green fluorescence allowing the use of standard FITC filter sets and co-staining with red fluorescent dyes. The stain can be used in low concentrations in the nanomolar range and shows high labeling potential with low background staining. Because the stain does not elicit cell toxicity, the Chromeo LT Live Cell Membrane Stain efficiently stains living cells, enabling long-term (LT) cell monitoring over a period of several days.

In addition to staining live cells, the Chromeo LT Live Cell Membrane Staining Kit can be used to label lipid structures in paraformaldehyde-fixed cells or paraffin-embedded tissue.

product	format	catalog no.
Chromeo™ LT Live Cell Membrane Staining Kit	1 kit	15007

* The Chromeo™ LT Live Cell Membrane Staining Kit is manufactured under license by OnkoTec GmbH.

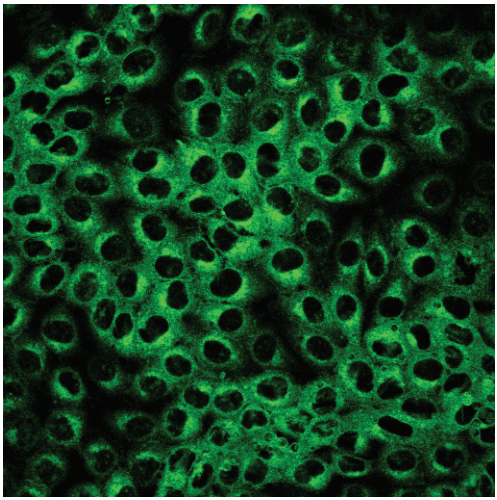

Figure 1: Staining of live NRK cells with the Chromeo LT Live Cell Membrane Stain.

NRK cells were plated in 3 cm cell culture dishes and washed once with pre-warmed PBS before adding 30 nM Chromeo LT Live Cell Membrane staining solution. After incubation for 10 minutes at 37°C, cells were washed once with pre-warmed PBS and were imaged in PBS with a Nikon CLSM (60x objective).

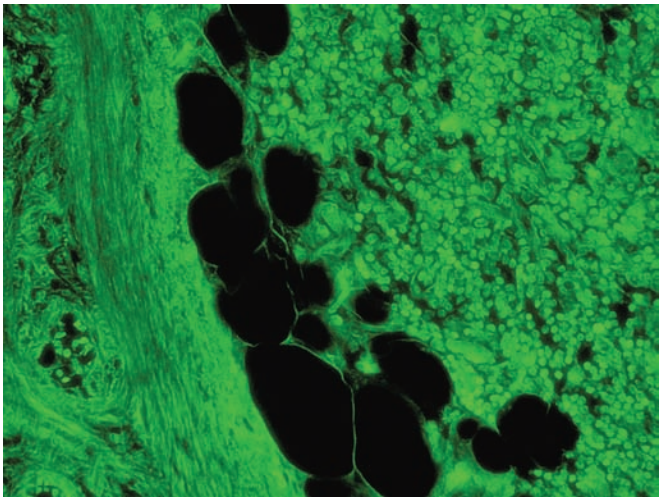

Figure 2: Staining of Formalin-Fixed Paraffin-Embedded (FFPE) epithelial carcinoma with the Chromeo LT Live Cell Membrane Stain.

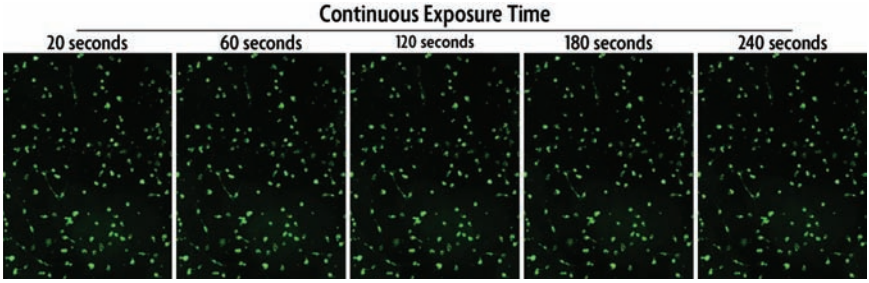

Figure 3: Photobleaching analysis of Chromeo LT Live Cell Membrane Stain.

HeLa cells were stained with 30 nM Chromeo LT Live Cell Membrane staining solution and washed once with pre-warmed PBS and illuminated continuously with a Zeiss Axiovert 200 (10x objective). Images were captured every 20 seconds; shown above are those from 20, 60, 120, 180 and 240 seconds.

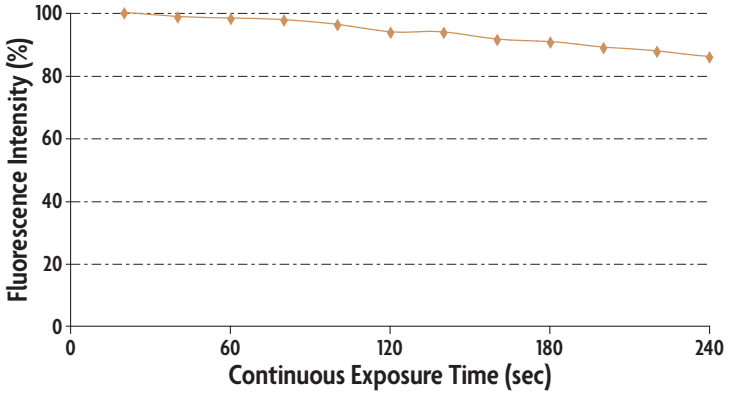

Figure 4: Statistical analysis of the photobleaching experiment shown in Figure 3.

Fluorescence intensities of the images shown in Figure 3 (above) were quantified using the ImageJ program.

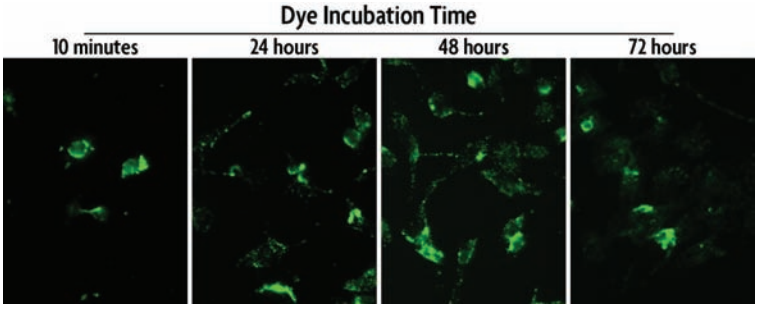

Figure 5: Long-term imaging of HeLa cells stained with the Chromeo LT Live Cell Membrane Staining Kit.

HeLa cells were seeded in 3 cm cell culture dishes and washed once with pre-warmed PBS before adding 30 nM Chromeo LT Live Cell Membrane staining solution. After incubation for 10 minutes at 37°C, cells were washed once with pre-warmed PBS and either imaged immediately in PBS with a Zeiss Axiovert 200 (40x objective), or incubated in culture medium for an additional 24, 48 or 72 hours at 37°C before imaging.

Kit Components and Storage

The Chromeo™ LT Live Cell Membrane Staining Kit contains 4 vials of lyophilized fluorescent Chromeo LT Live Cell Membrane Stain. Each vial contains stain sufficient to produce 30 ml of Staining Solution after reconstitution of the lyophilized complex.

Store the vials at 4°C for 12 months protected from light.

Additional materials required

- Tissue culture supplies
- Cell culture medium
- PBS
- Distilled water, sterile
- Fluorescence instrumentation

Protocols

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

Note: Dilutions and quantities provided are guidelines. You may need to vary the conditions to obtain optimal results for your specific cell type or culture equipment.

A. Preparation of the Stock Solution

To prepare the Stock Solution, add 2 ml of distilled water into one vial containing the Chromeo LT Live Cell Membrane Stain, vortex carefully and incubate for 10 minutes at 37°C protected from light. To completely dissolve the fluorescent complex, incubate the vial for 10 minutes in an ultrasonic bath. This procedure results in a 15X Stock Solution (0.45 µM).

The Stock Solution is stable in the dark for one week when stored at 4°C and stable for 6 months when stored at -20°C. Before using Stock Solution that has been stored, vortex it for 30 seconds to resuspend precipitated fluorescent stain.

To avoid repeated freeze-thaw cycles, freeze the Stock Solution in appropriate aliquots.

B. Preparation of the Staining Solution

Prepare fresh Staining Solution every time you perform a new staining experiment.

To prepare the Staining Solution, dilute the appropriate volume of Chromeo LT Live Cell Membrane Stain Stock Solution in PBS. We recommend to dilute the Stock Solution 1:15 in PBS, resulting in a 30 nM Staining Solution. If you are performing live cell staining, the Staining Solution (and regular PBS buffer for washing steps) should be warmed to 37°C before beginning the procedure.

For some cell types or special applications, other concentrations of the fluorescent stain may be necessary; dilute the Staining Solution in a suitable manner.

C. Staining of Live Cells

Note: To ensure the quality of the staining and to maintain the stability of the dye, minimize light exposure of the stained cells as much as possible.

1. Grow the cells to the desired confluence on a suitable coverglass or in cell culture dishes.
2. Prior to staining, rinse the cells once with pre-warmed PBS.
3. Add the Staining Solution to the cells and incubate for 10 minutes at 37°C protected from light. To stain cells grown in a 6-well plate, 0.5 ml of Staining Solution per well is sufficient for effective membrane labeling.
4. Rinse cells once with pre-warmed PBS.
5. Image the cells in PBS or in a cell culture medium that does not contain phenol red in order to avoid background fluorescence.
6. If the cells will not be imaged immediately, add fresh cell culture medium and return the cells to the incubator.

   Note: Due to its high affinity for lipid structures, Chromeo LT Live Cell Membrane Stain is suitable for long-term live cell imaging allowing the cells to be imaged for several days after being stained. However, you should take into consideration that the fluorescence intensity decreases with time as the cells divide and the dye is distributed between daughter cell membranes.

D. Staining of Fixed Cells

Caution: The dye is soluble in methanol, ethanol and other organic solvents, which results in complex dissociation and severely reduced staining capability.

Chromeo LT Live Cell Membrane Stain is compatible with cell fixation using 3.7% paraformaldehyde (PFA). To obtain bright fluorescent staining, stain the cells after the fixation procedure.

1. Grow the cells to the desired confluence on a suitable coverglass or in cell culture dishes.
2. Prior to fixation, rinse the cells once with pre-warmed PBS.
3. Add the 3.7% PFA solution to the cells and incubate for 15 minutes at room temperature.
4. Rinse the cells twice with PBS.
5. Add the Staining Solution to the cells and incubate for 10 minutes at room temperature protected from light.
6. Rinse the cells twice with PBS.
7. Mount the cells in appropriate mounting media.

Note: Chromeo LT Live Cell Membrane Stain is compatible with co-staining with other fluorescent dyes like Hoechst, LysoTracker® Red DND-99 (Invitrogen), MitoTracker® Orange CMTMRos (Invitrogen) or BODIPY® 558/568 C12 (Invitrogen).

E. Staining of Paraffin Sections

1. De-paraffinize and re-hydrate paraffin-embedded tissue sections with:

   1 x 10-15 minutes xylene
   1 x 2-5 minutes ethanol (100%)
   1 x 2-5 minutes ethanol (80%)
   1 x 2-5 minutes ethanol (70%)

2. Wash sections with PBS for 5 minutes and incubate with 1-2 ml Staining Solution for 20-30 minutes.
3. Mount sections in appropriate mounting media.

F. Image Acquisition

Analyze the stained cells by fluorescence microscopy. To detect Chromeo LT Live Cell Membrane Stain, a standard FITC filter set can be used. In general, the broad absorption and emission peaks of the fluorescent complex (excitation from 420 nm to 490 nm/emission from 500 nm to 540 nm) provide flexibility for excitation and detection, and enable you to choose from several commonly used filter sets.

For long-term labeling of living cells, we recommend choosing a short exposure time for each acquisition to prevent photobleaching of the fluorescent complex.

Appendix

Section A. Troubleshooting Guide

Problem/question	Possible cause	Recommendation
Staining differs between different cell types	The amount and the morphology of the membranes differ between the cell types.	
	The amount of stain necessary to get optimal membrane staining might vary between different cell types.	Optimize the staining experiment by using different concentrations of the Staining Solution and longer incubation times.
Speckled background staining	The Staining Solution may contain undissolved stain complexes.	Re-incubate the Stock Solution for 5-10 minutes in an ultrasonic bath at 50°C. Then, prepare fresh Staining Solution.
Lack of staining in fixed tissues	Slides have been fixed with methanol, which may cause dissociation of the fluorescent complex.	Use 3.7% paraformaldehyde (PFA) for fixation.

Section B. Related Products

Fluorescent Cell Stains	Format	Catalog No.
LavaCell™ Live Cell Membrane Staining	200 µg	15004
Chromeo™ Red Fluorescent Fixed Cell Staining Kit	1 kit	15006
Chromeo™ Live Cell Mitochondrial Staining Kit	1 kit	15005

Fluorescent Antibody/Protein Labeling	Excitation / Emission	Format	Catalog No.
Chromeo™ 488 Antibody Labeling Kit	488 nm / 517 nm	1 kit	15090
Chromeo™ 494 Antibody Labeling Kit	494 nm / 628 nm	1 kit	15091
Chromeo™ 546 Antibody Labeling Kit	545 nm / 561 nm	1 kit	15092
Chromeo™ 642 Antibody Labeling Kit	642 nm / 660 nm	1 kit	15093

Fluorescent Secondary Antibodies	Excitation / Emission	Format	Catalog No.
Chromeo™ 488 Goat anti-Mouse IgG	498 nm / 524 nm	1 mg	15031
Chromeo™ 488 Goat anti-Rabbit IgG	498 nm / 524 nm	1 mg	15041
Chromeo™ 494 Goat anti-Mouse IgG	489 nm / 624 nm	1 mg	15032
Chromeo™ 494 Goat anti-Rabbit IgG	489 nm / 624 nm	1 mg	15042
Chromeo™ 505 Goat anti-Mouse IgG	514 nm / 530 nm	1 mg	15030
Chromeo™ 505 Goat anti-Rabbit IgG	514 nm / 530 nm	1 mg	15040
Chromeo™ 546 Goat anti-Mouse IgG	550 nm / 567 nm	1 mg	15033
Chromeo™ 546 Goat anti-Rabbit IgG	550 nm / 567 nm	1 mg	15043
Chromeo™ 642 Goat anti-Mouse IgG	647 nm / 666 nm	1 mg	15034
Chromeo™ 642 Goat anti-Rabbit IgG	647 nm / 666 nm	1 mg	15044
ATTO 594 Goat anti-Mouse IgG	601 nm / 627 nm	250 µl	15037
ATTO 594 Goat anti-Rabbit IgG	601 nm / 627 nm	250 µl	15047
ATTO 647N (STED) Goat anti-Mouse IgG	644 nm / 669 nm	250 µl	15038
ATTO 647N (STED) Goat anti-Rabbit IgG	644 nm / 669 nm	250 µl	15048
ATTO 655N (STED) Goat anti-Mouse IgG	663 nm / 684 nm	250 µl	15039
ATTO 655N (STED) Goat anti-Rabbit IgG	663 nm / 684 nm	250 µl	15049
ATTO 532 (GSD) Goat anti-Mouse IgG	534 nm / 560 nm	250 µl	15070
ATTO 532 (GSD) Goat anti-Rabbit IgG	534 nm / 560 nm	250 µl	15072
Rhodamine 6G (GSD) Goat anti-Mouse IgG	508 nm / 558 nm	250 µl	15074
Rhodamine 6G (GSD) Goat anti-Rabbit IgG	508 nm / 558 nm	250 µl	15076

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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