molecular probes®
by life technologies™

CellMask™ Plasma Membrane Stains

Catalog numbers C10045, C10046, C37608

Table 1 Contents and storage

Material	Amount	Storage	Stability
CellMask™ Orange plasma membrane stain (C10045), CellMask™ Deep Red plasma membrane stain (C10046), or CellMask™ Green plasma membrane stain (C37608)	100 µL	• <–20°C • Desiccate • Protect from light	When stored as directed, the product is stable for at least 1 year
Number of assays: Sufficient material is supplied for 100 assays, based on the following protocol.			
Approximate fluorescence excitation/emission maxima: CellMask™ Orange: 554/567 nm; CellMask™ Deep Red: 649/666 nm; CellMask™ Green: 522/535 nm.			

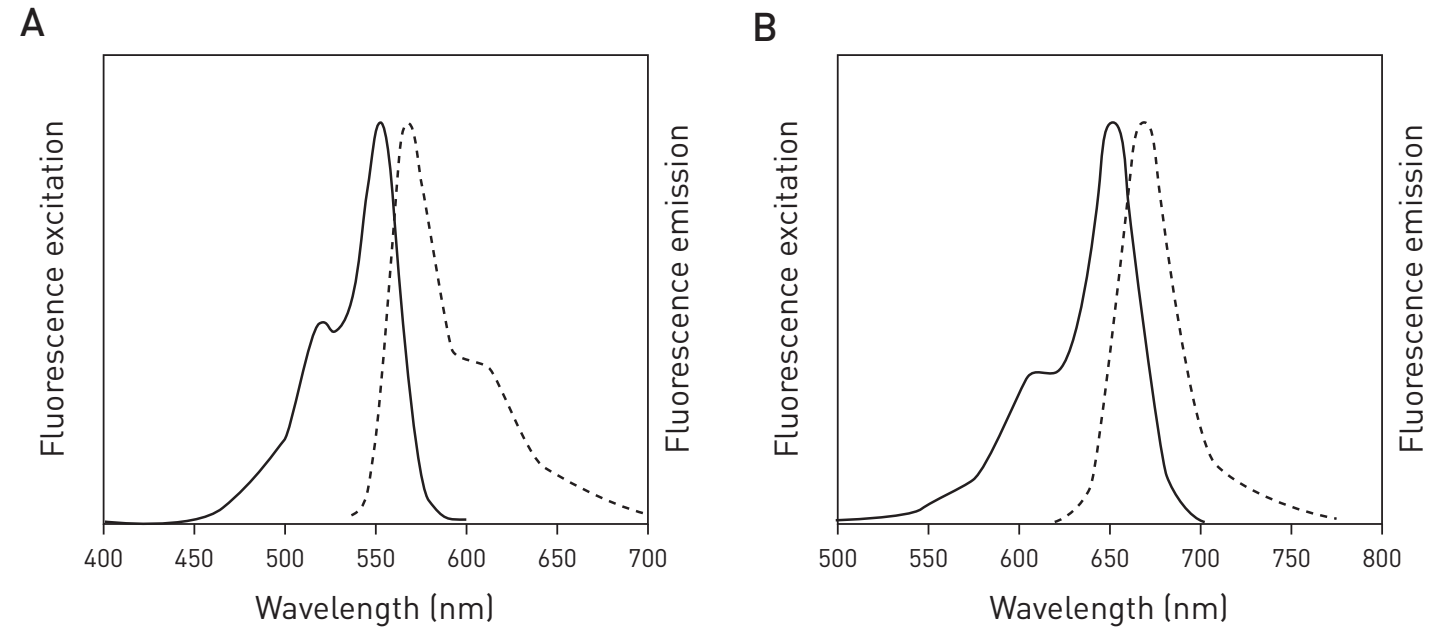
Introduction

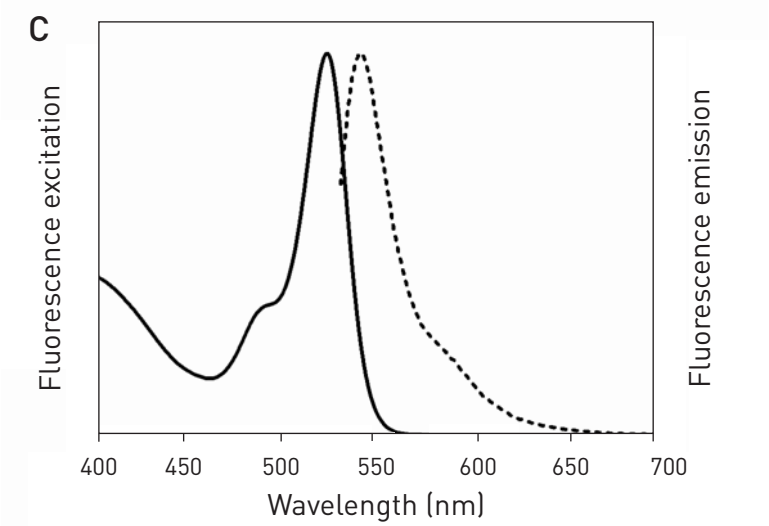
The plasma membrane is a convenient marker of cell boundaries and as such, a number of probes have been utilized for this purpose. Typically, dyes of a lipophilic nature are used as plasma membrane stains; however, they internalize rapidly offering a very narrow window for imaging. Fluorescently labeled lectins, such as wheat germ agglutinin, have also been employed as plasma membrane stains. Conjugated lectins depend on cell surface sugars for staining and as a result, stain inconsistently with variation across cell types. Robust plasma membrane staining is important for a range of applications including translocation assays, plasma membrane dynamics, and as a general tool for cell identification in traditional and automated imaging and analysis.

CellMask™ Orange, Deep Red, and Green plasma membrane stains deliver uniform staining of the plasma membrane across a wide variety of mammalian cell types and are slow to internalize, particularly compared to traditional approaches such as DiI, DiO, and labeled wheat germ agglutinin. The CellMask™ plasma membrane stains provide excellent and rapid plasma membrane staining in live cells for 30–90 minutes depending on the cell type and experimental conditions. The CellMask™ plasma membrane stains are amphipathic molecules providing a lipophilic moiety for excellent membrane loading and a negatively charged hydrophilic dye for "anchoring" of the probe in the plasma membrane. While the CellMask™ plasma membrane stains provide ample opportunity for live cell imaging, the staining pattern is also maintained after fixation with formaldehyde, enabling more multiparametric imaging options. However, the staining with CellMask ™ stains does not survive detergent extraction and therefore cannot be used in conjunction with probes that require permeabilization.

The CellMask™ plasma membrane stains provide robust and flexible staining in live and fixed cells useful for traditional fluorescence microscopy applications, automated imaging, and analysis (Figure 1, page 2).

Figure 1. The fluorescence emission spectra of CellMask™ Orange (panel A), Deep Red (panel B), and Green (panel C) plasma membrane stains.





Before you begin

Materials required but not provided

- Appropriate cell culture medium
- (*Optional*) Dulbecco's Phosphate Buffered Saline containing calcium and magnesium
- (*Optional*) 3.75% formaldehyde in buffer or media for fixation
- (*Optional*) Mounting medium if samples will be retained

Cautions DMSO is hazardous; avoid contact with skin and eyes and do not swallow. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials.

No data are available addressing the toxicity data or health hazards of the CellMask™ plasma membrane stains. Because this reagent binds to plasma membrane, handle with appropriate care and take the same safety precautions as for other chemicals with unknown toxicity. Dispose of the stains in compliance with all pertaining local regulations.

Experimental protocol

Use the following staining protocol with the CellMask™ plasma membrane stains as a guideline for plasma membrane staining of live, adherent cultured cells on coverslips. Optimal conditions may vary depending upon the characteristics of the cells used. This staining protocol was tested with various common cell lines such as mouse embryonic fibroblast and human embryonic kidney adherent cells but has been optimized using highly confluent adherent human osteosarcoma cells.

We recommend the use of physiologically relevant buffer such as such as Live Cell Imaging solution (Cat. no. A14291DJ).

Staining

1.1 Prepare a *fresh* working solution of the CellMask™ plasma membrane stain in warm physiologically relevant buffer from the provided 1000X concentrated stain solution. For example, to prepare 10 mL of 1X working solution, add 10 µL of the stain to 10 mL of physiologically relevant media.

Note: The optimal concentration may vary depending on cell type and staining conditions. We have successfully used 0.5X–1.5X working solutions with different cell types.

1.2 Grow cells on coverslips inside a tissue culture dish with the appropriate culture medium.

Note: If you wish to use suspension cells, grow the suspension cells in the appropriate culture medium to the desired confluency and then spot the suspension cells on poly D-Lysine coated coverslips. Use the following staining protocol for adherent or suspension cells.

1.3 When cells have reached the desired confluency, remove the coverslip from the culture medium, wash with physiologically relevant buffer, such as Live Cell Imaging solution, and quickly submerge the coverslip in the staining solution from step **1.1** for 5–10 minutes at 37ºC.

If fixation is desired, proceed to "**(*Optional*) Fixation after staining**" on page 4.

1.4 Remove the staining solution and rinse the coverslip with physically relevant buffer three times.

1.5 Mount the coverslip and image immediately.

(*Optional*) Fixation after staining

2.1 Remove the staining solution and fix the cells after staining (step **1.3**) with warm 3.75% formaldehyde in buffer or media and incubate at 37ºC for 5–10 minutes.

2.2 Rinse the coverslip three times with buffer.

2.3 Mount the coverslip and image immediately or within 24 hours (if sample is mounted in an antifade reagent, such as ProLong® Gold antifade reagent, Cat. no. P36930).

Imaging

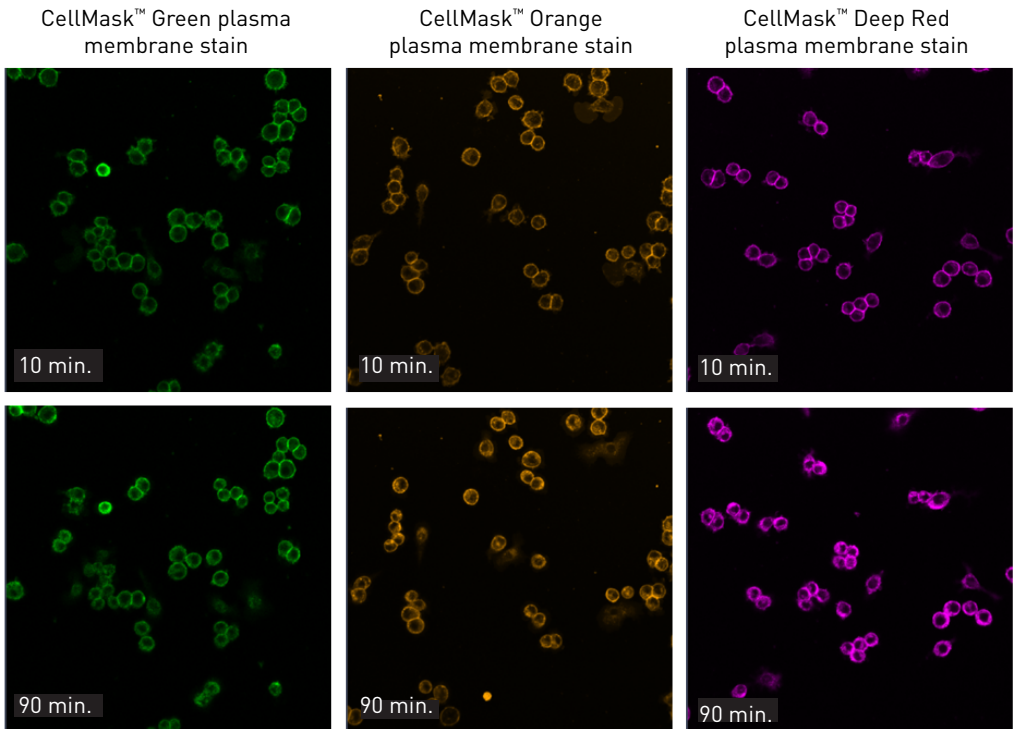
Image the samples immediately, or within 24 hours of staining for fixed samples mounted in antifade reagent, according to Table 2 (see Figure 2).

Table 2 Imaging parameters

Stain	Filter set	Excitation/Emission maxima (nm)
CellMask™ Orange Plasma Membrane stain	Standard TRITC/RFP	554/567
CellMask™ Deep red Plasma Membrane stain	Standard CY5/Deep red	649/666
CellMask™ Green Plasma Membrane stain	Standard FITC/GFP	522/535

Note: If you used suspension cells spotted on poly D-Lysine coated coverslips for the staining protocol, the background may be high but the signal-to-noise ratio is still very good.

Figure 2. Human macrophage MMM cells labeled with a 1X solution of CellMask™ Green plasma membrane stain (left images), CellMask™ Orange plasma membrane stain (middle images), or CellMask™ Deep Red plasma membrane stain (right images) using the preceding protocol. Cells were imaged using appropriate filters immediately after washing (upper panel), and after 90 minutes (lower panel). Imaging was performed with a confocal microscope using a 20X objective. The images show a distinct plasma membrane staining, with minimum internalization 90 minutes after the removal of the staining solution.



Troubleshooting

Observation	Cause	Solution
No signal	Incorrect filters or set up	Make sure you are using an appropriate filter set to detect the signals. See the "Product list" for the recommended filter set for use with CellMask™ stains.
	Optimize staining conditions	Depending on the cell line used, you may need to increase the staining time or concentration to obtain the desired signal intensity.
High background	Sample dried during processing	Avoid specimen drying as this can cause high levels of non-specific background and autofluorescence. Use appropriate solution volumes and containers to make sure the coverslip is completely covered with solution during the staining and washing steps.
	Optimize staining conditions	Decrease the stain concentration, and/or increase the number and/or duration of wash steps. Perform staining in physiologically appropriate buffer instead of culture medium.

Product list Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
C10045	CellMask™ Orange plasma membrane stain *5 mg/mL in DMSO*	0.1 mL
C10046	CellMask™ Deep Red plasma membrane stain *5 mg/mL in DMSO*	0.1 mL
C37608	CellMask™ Green plasma membrane stain *5 mg/mL in DMSO*	0.1 mL
Related Products		
P36930	ProLong® Gold antifade reagent	10 mL
A14291DJ	Live Cell Imaging solution	500 mL

*Semrock® BrightLine® filters are supplied by Semrock, Inc. (www.semrock.com) and available from Life Technologies™.

Purchaser notification

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Additional international offices are listed at **www.lifetechnologies.com**

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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