CellMask™ Plasma Membrane Stains

Catalog Numbers C10045, C10046, C37608, and C56129

Pub. No. MAN0029181 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The plasma membrane is a convenient cell boundary marker commonly used for probes. Lipophilic dyes are typically used as plasma membrane stains, but they can internalize rapidly and offer a 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 because of variations 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, Green, and NIR plasma membrane stains deliver uniform staining of the plasma membrane in live and fixed cells across various mammalian cell types. These stains are slow to internalize when compared to traditional approaches (examples, DiI, DiO, labeled wheat germ agglutinin) and provide rapid plasma membrane staining in live cells for 30–90 minutes depending on the cell type and experimental conditions. The 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. Although the stains provide ample opportunity for live cell imaging, the staining pattern is also maintained after formaldehyde fixation, enabling more multiparametric imaging options. However, this CellMask™ staining does not survive detergent extraction and, therefore, cannot be used with probes requiring permeabilization. CellMask™ stains are useful for traditional fluorescence microscopy applications, automated imaging, and analysis (Figure 1).

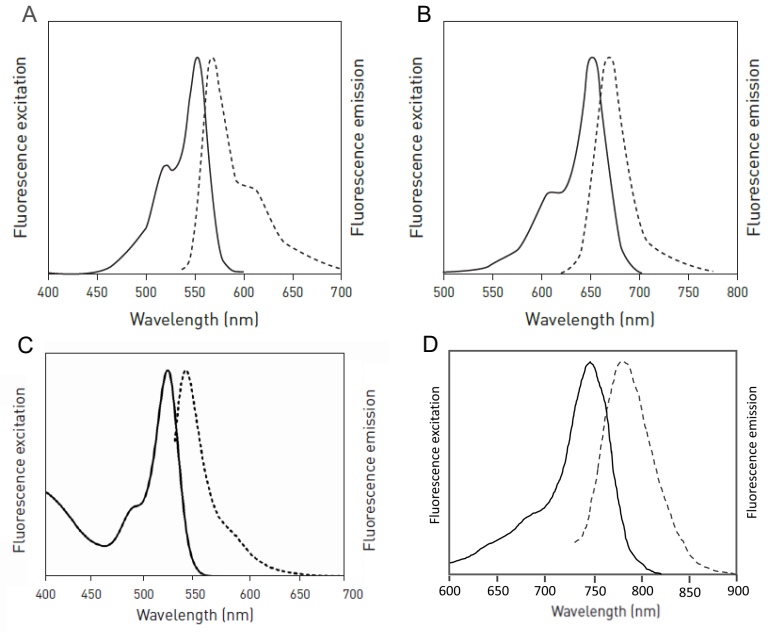

Figure 1 Fluorescence emission spectra of (A) CellMask™ Orange, (B) Deep Red, (C) Green, and (D) NIR plasma membrane stains.

Contents and storage

Item	Cat. No.	Amount [1]	Approx. fluorescence excitation/emission (Ex/Em) maxima	Storage[2]
CellMask™ Orange Plasma Membrane Stain	C10045	100 µL	554/567 nm	• Store at or below –20°C • Desiccate • Protect from light
CellMask™ Deep Red Plasma Membrane Stain	C10046	100 µL	649/666 nm	
CellMask™ Green Plasma Membrane Stain	C37608	100 µL	522/535 nm	
CellMask™ NIR Plasma Membrane Stain	C56129	100 µL	748/780 nm	• Store at room temperature • Desiccate • Protect from light

[1] Sufficient material for 100 assays when used in the following protocols.
[2] Product is stable for at least 1 year when stored as directed.

Required materials 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

Stain plasma membranes

Use this staining protocol with the CellMask™ plasma membrane stains as a guideline for plasma membrane staining of live, adherent cultured cells on coverslips. Optimal conditions can vary depending on 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).

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 can vary depending on cell type and staining conditions. We have successfully used 0.5X–1.5X working solutions with different cell types.

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

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

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 for 5–10 minutes at 37°C.

   To perform fixation, proceed to “(Optional) Fix after staining” on page 2.

4. Remove the staining solution and rinse the coverslip with physically relevant buffer 3 times.

5. Mount the coverslip and image immediately.

(Optional) Fix after staining

1. Remove the staining solution and fix the cells after staining (step 3) with warm 3.75% formaldehyde in buffer or media and incubate at 37°C for 5–10 minutes.

2. Rinse the coverslip with buffer 3 times.

3. Mount the coverslip and image immediately or within 24 hours if sample is mounted in an antifade reagent such as ProLong™ Gold Antifade Mountant (Cat. No. P36930).

Image samples

IMPORTANT! Image samples immediately or within 24 hours of staining for fixed samples mounted in antifade reagent according to Table 1.

Table 1 Imaging parameters.

Stain	Filter set	Ex/Em maxima
CellMask™ Orange Plasma Membrane Stain	Standard TRITC/RFP	554/567 nm
CellMask™ Deep Red Plasma Membrane Stain	Standard CY5/Deep red	649/666 nm
CellMask™ Green Plasma Membrane Stain	Standard FITC/GFP	522/535 nm
CellMask™ NIR Plasma Membrane Stain	Standard CY7	748/780 nm

Note: If using suspension cells spotted on D-Lysine-coated coverslips for the staining protocol, the background signal can be higher, but the signal-to-noise ratio is still optimal.

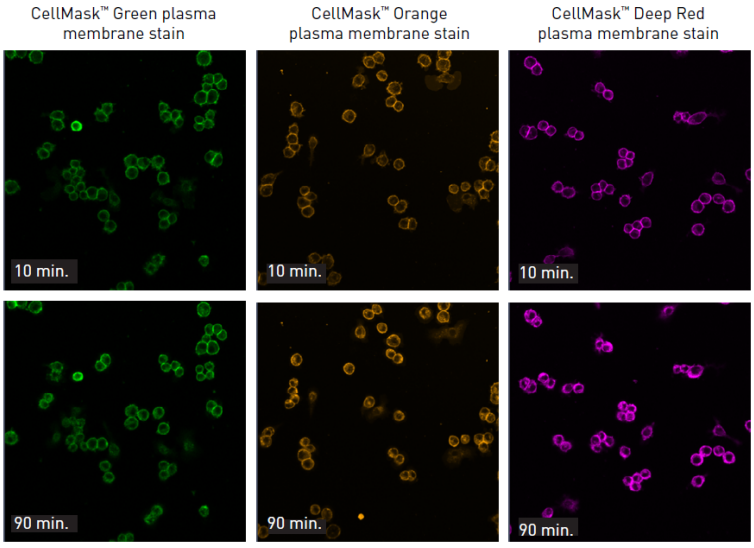

Figure 2 Human macrophage MMM cells labeled with a 1X solution of CellMask™ Green Plasma Membrane Stain, CellMask™ Orange Plasma Membrane Stain, or CellMask™ Deep Red Plasma Membrane Stain using the preceding protocol. Cells were imaged using appropriate filters 10 minutes after washing (top) and after 90 minutes (bottom). 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	Possible cause	Recommended action
No signal	Incorrect filters or set up.	Ensure an approriate filter set is used to detect the signals. See “Image samples” on page 3 for the recommended filter set for each stain.
	Optimize staining conditions.	Increase the staining time or concentration for each cell line to obtain the desired signal intensity.
High background	Sample dried during processing.	Avoid specimen drying because this can cause high levels of nonspecific background and autofluorescence.
		Use appropriate solution volumes and containers to ensure 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 length of wash steps.
		Perform staining in physiologically appropriate buffer instead of culture medium.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A.0	10 March 2023	New document for CellMask™ Plasma Membrane Stains.