SZABO SCANDIC

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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Product Information

CytoLiner™ Fixed Cell Membrane Stains

Kit Contents

Component	250 Labelings	1000 Labelings
CytoLiner™ Fixed Cell Membrane Dye, 500X in DMSO	Component A 50 uL	Component A 200 uL
CytoLiner™ Fixed Cell Staining Buffer, 100X	99890-1ML 1 mL	99890-1ML 1 mL

Storage and Handling

Store at 4°C. Protect dye component from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Prepare 1X CytoLiner™ Fixed Cell Staining Buffer by diluting the 100X buffer with dH_2O at a ratio of 1:100. For example, add 10 uL of 100X CytoLiner™ Fixed Cell Staining Buffer to 990 uL of dH_2O. Scale volumes as needed. 1X Staining Buffer is stable at 4°C or room temperature. Vortex to mix well.

Prepare working solution of CytoLiner™ Dye diluted in 1X Staining Buffer within 10 minutes of use.

Product Description

CytoLiner™ Fixed Cell Membrane Dyes are novel lipophilic fluorescent dyes developed specifically for staining fixed cells. While classic lipophilic membrane dyes like DiI can be used to stain formaldehyde-fixed cells, staining can be highly variable due to the poor solubility of the dyes. CytoLiner™ Dyes have optimized chemical structures to balance solubility with lipophilicity to permit selective staining of the plasma membrane in fixed and mildly permeabilized cells. CytoLiner™ staining is compatible with formaldehyde fixation and mild detergent permeabilization before staining. Staining can tolerate blocking agents used during immunofluorescence staining protocols, allowing subsequent staining with antibodies and other probes. The dyes also are compatible with poly-L-lysine coated cultureware and Transwell® permeable filter supports.

CytoLiner™ Fixed Cell Membrane Stains are part of a family of unique and innovative cell membrane stains: CellBrite® Steady, for long-term cell surface staining of live cells; CellBrite® Fix and MemBrite® Fix, for fixable staining; and a wide selection of CF® Dye-conjugated lectins for staining cell surface glycoproteins. See Related Products for more information.

Considerations for Staining with CytoLiner™ Dyes

- CytoLiner™ staining can be performed on cells fixed with formaldehyde or paraformaldehyde (PFA). Staining is not compatible with cells fixed using solvents like methanol or acetone, or with paraffin-embedded samples, because these treatments will remove the lipids required for CytoLiner™ staining.
- CytoLiner™ Dyes will stain tissue cryosections, but because plasma membranes are compromised by freezing and sectioning, the staining is not selective for the plasma membrane but will also stain intracellular structures.
- CytoLiner™ staining tolerates mild permeabilization before staining. We recommend using PBS/0.1% Triton X-100 for 10 minutes at room temperature. We have found that this method of permeabilization generally improves uniformity and brightness of staining compared to PFA fixation alone. Longer permeabilization times will reduce plasma membrane staining. Permeabilization conditions may require optimization for different cell types or culture formats.
- For best results, dilute CytoLiner™ Dyes in 1X CytoLiner™ Staining Buffer for staining. Other buffer such as PBS may be used, but the staining may be less specific.
- For co-staining with CytoLiner™ Dyes and antibody conjugates, we recommend staining with CytoLiner™ Dye first, then blocking with 2% fish gelatin in PBS, followed by antibody incubation in the same buffer. Biotium's 10X Fish Gelatin Blocking Agent (Cat. No. 22016) diluted to 1X or 2X in PBS may be used. PBS with 1% BSA also is compatible with CytoLiner™ Dyes. Blocking with serum is not recommended.
- Dye concentration and staining time may require optimization for different cell types and imaging systems.
- After initial cell permeabilization and CytoLiner™ staining, detergent should not be used in any blocking, antibody incubation, or wash steps because this will extract the CytoLiner™ Dyes.
- CytoLiner™ staining cannot tolerate mounting medium or clearing agents. Cells should be imaged in PBS.
- CytoLiner™ Dyes can be co-incubated with select NucSpot® Nuclear Stains (Cat. No. 41033-41038, 41040; see Related Products). For co-staining with other nuclear stains or other fluorescent probes, we recommend staining with CytoLiner™ Dye first, and then staining with the nuclear stain or fluorescent probe.

Table 1. CytoLiner™ Fixed Cell Membrane Dyes

Cat. No.	CytoLiner™ Dye	Abs/Em	Detection Channel
30131A	CytoLiner™ 410/450 Fixed Cell Membrane Dye	406/446 nm	DAPI / Pacific Blue™
30132A	CytoLiner™ 495/510 Fixed Cell Membrane Dye	492/510 nm	FITC
30133A	CytoLiner™ 570/590 Fixed Cell Membrane Dye	573/592 nm	Cy®3 / TRITC
30134A	CytoLiner™ 650/675 Fixed Cell Membrane Dye	647/674 nm	Cy®5
30135A	CytoLiner™ 680/710 Fixed Cell Membrane Dye	682/707 nm	Cy®5.5

Considerations for Staining with CytoLiner™ Dyes (Cont. from page 1)

- CytoLiner™ Dyes will stain live cell membranes, but background on culture surfaces tends to be higher with live cell staining compared to staining after PFA fixation. For imaging the cell surface in live cells, we recommend CellBrite® Steady. For staining live cell surface followed by fixation, we recommend CellBrite® Fix, MemBrite® Fix, or CF® Dye Lectin Conjugates (note that lectin staining may vary between different cell types).
- CytoLiner™ staining is compatible with poly-L-lysine coated culture surfaces and Transwell® permeable supports. For best results, confocal microscopy is recommended for imaging fluorescent staining of Transwell® supports to avoid background from the filter material.
- After fixed cells are stained, CytoLiner™ signal is stable. Stained cells may be stored in PBS at 4°C, protected from light, for several days or longer prior to imaging. Coverslips mounted in buffer should be sealed and imaged as soon as possible to avoid drying out.
- CytoLiner™ Dyes have been used to stain the outer membranes of MCF-7 cell spheroids. However, penetration of the dyes to interior membranes was variable. Staining of three-dimensional cultures may require optimization of fixation and permeabilization conditions.
- Confocal microscopy allows selective imaging of cell boundaries using membrane dyes because it detects fluorescence from a narrow plane of focus. When epifluorescence microscopy is used to image membrane dyes, staining will appear more diffuse across the cell because fluorescence from multiple planes of the cell membrane is detected.

Procedures for Fixation and Staining

Materials required but not provided

- HBSS with calcium/magnesium (recommended for adherent cells)
- 1X PBS
- 4% Paraformaldehyde (PFA) in PBS (Cat. No. 22023 or equivalent)
- PBS + 0.1% Triton® X-100, or Biotium's Permeabilization Buffer (Cat. No. 22016)

Staining procedure for adherent cells

1. Remove the culture medium from live cells.
2. Rinse cells twice in HBSS with calcium/magnesium.
3. Fix cells with 4% PFA in PBS for 15 minutes at room temperature.

   Note: Fixation concentration, time, and temperature may require optimization for different cell types or co-stains.
4. Rinse cells three times with PBS.
5. Permeabilize the cells in PBS with 0.1% Triton® X-100 for 10 minutes at room temperature.

   Note: Permeabilization time, temperature, and detergent concentration may require optimization for different cell types or culture conditions. Using higher concentrations of Triton® X-100 or permeabilizing for more than 10 minutes at room temperature may result in dimmer and less specific staining.
6. Rinse the cells three times with PBS.
7. Prepare 1X CytoLiner™ Staining Buffer as described in the Storage and Handling section.

   Note: Staining may be done in other buffers such as PBS, but the staining may be less specific. For best results, use 1X CytoLiner™ Staining Buffer.
8. Prepare CytoLiner™ Dye staining solution by diluting the 500X CytoLiner™ Dye (Component A) in 1X CytoLiner™ Staining Buffer at a ratio of 1:500 and immediately vortex to mix completely. For example, add 1 uL of dye to 500 uL of 1X Buffer.

   Note: Prepare the staining solution within 10 minutes of use. Prepare the staining solution in a tube and mix well before adding to cells. Do not add concentrated CytoLiner™ Dye directly to cells in buffer because this may result in uneven staining. Dye concentration may require optimization for different cell types.
9. Remove the PBS from the cells and add the 1X staining solution. You should use enough volume to cover the cells completely.
10. Incubate for 10 minutes at room temperature, protected from light.

    Note: Staining time may require optimization for different cell types.

11. Remove the staining solution and rinse the cells three times with PBS.
12. Proceed with imaging (see Table 1 for detection channels), or continue to blocking and staining with antibodies or other probes.

    Note: After the initial permeabilization step and CytoLiner™ staining, all buffers used for blocking, antibody incubation, and washing must be detergent-free. See Considerations for Staining with CytoLiner™ Dyes above for blocking buffer recommendations.

Staining procedure for cells in suspension

Note: Scale volumes up proportionally when staining higher cell numbers.

1. Transfer up to 1 x 10^6 cells to a microcentrifuge tube.
2. Pellet the cells by centrifuging at 350 x g for three minutes.
3. Remove supernatant and resuspend the pellet in 500 uL of PBS to wash.
4. Pellet the cells and repeat step 3 for two washes total.
5. Pellet the cells and resuspend in 100 uL of 4% paraformaldehyde in PBS. Incubate for 15 minutes at room temperature.

   Note: Fixation time and temperature may require optimization for different cell types.
6. Pellet the cells and remove the supernatant. Wash twice with 500 uL of PBS as in step 3.
7. Pellet the cells and resuspend in 100 uL of PBS with 0.1% Triton® X-100. Incubate for 10 minutes at room temperature.

   Note: Permeabilization time, temperature, and detergent concentration may require optimization for different cell types or culture conditions. Using higher concentrations of Triton® X-100 or permeabilizing for more than 10 minutes at room temperature may result in dimmer and less specific staining.
8. Pellet the cells and wash twice in 500 uL PBS as in step 3.
9. Prepare 1X CytoLiner™ Staining Buffer as described in the Storage and Handling section.

   Note: Staining may be done in other buffers such as PBS, but the staining may be less specific. For best results, use 1X CytoLiner™ Staining Buffer.
10. Prepare CytoLiner™ Dye staining solution by diluting the 500X CytoLiner™ Dye (Component A) in 1X CytoLiner™ Fixed Cell Staining Buffer, at a ratio of 1:500. Vortex to mix immediately.

    Note: Use the staining solution within 10 minutes of preparation. Dye concentration may require optimization for different cell types.
11. Pellet cells and remove supernatant. Add 100 uL of CytoLiner™ Dye staining solution and resuspend cells by gently pipetting up and down.
12. Incubate for 10 minutes at room temperature, protected from light.

    Note: Staining time may require optimization for different cell types.
13. Pellet the cells and wash twice with 500 uL PBS as in step 3.
14. Resuspend the cells in 100 uL of PBS.
15. Proceed with imaging (see Table 1 for detection channels), or continue to blocking and staining with antibodies or other probes.

    Note: After the initial permeabilization step and CytoLiner™ staining, all buffers used for blocking, antibody incubation, and washing must be detergent-free. See Considerations for Staining with CytoLiner™ Dyes above for blocking buffer recommendations.

Related Products

Cat. No.	Product
22023	4% Paraformaldehyde in PBS
22016	Permeabilization Buffer
23005	CoverGrip™ Coverslip Sealant
22010	10X Fish Gelatin Blocking Agent
22011	Fish Gelatin Powder
22014	Bovine Serum Albumin, 30% Solution
22013	Bovine Serum Albumin Fraction V
30105-30109	CellBrite® Steady Membrane Staining Kits
30088-30090	CellBrite® Fix Membrane Stains
30092...30104	MemBrite® Fix Cell Surface Staining Kits
92230...92457	Mix-n-Stain™ CF® Dye Antibody Labeling Kits
29021...29059	Wheat Germ Agglutinin (WGA) Conjugates
29015...29058	Concanavalin A (Con A) CF® Dye Conjugates
29096-29101	Datura Stramonium Lectin (DSL) Conjugates
41033...41040	NucSpot® Nuclear Stains

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent stains for visualizing cell nuclei, membranes, mitochondria, or other organelles. Biotium also offers fluorescent CF® Dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

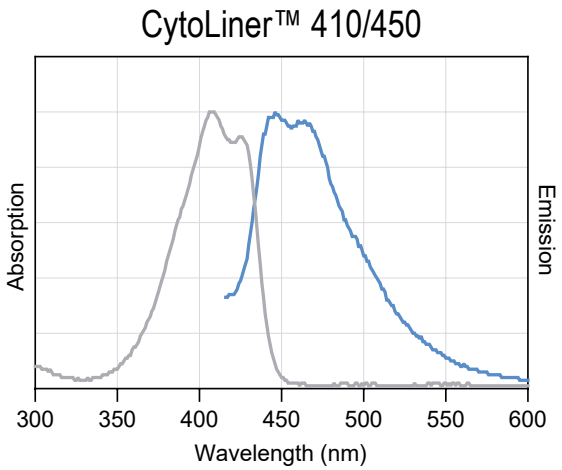

Figure 1. CytoLiner™ 410/450 absorbance and emission spectra.

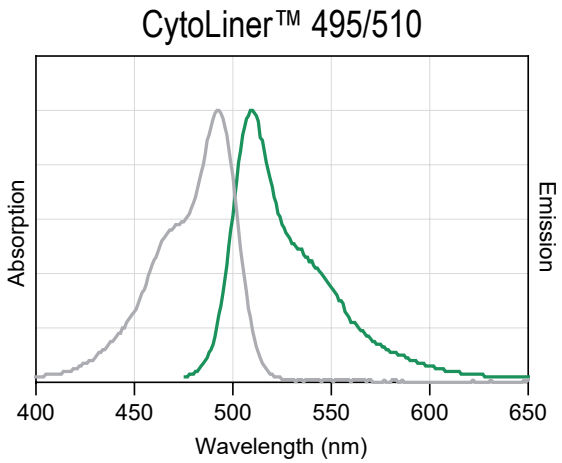

Figure 2. CytoLiner™ 495/510 absorbance and emission spectra.

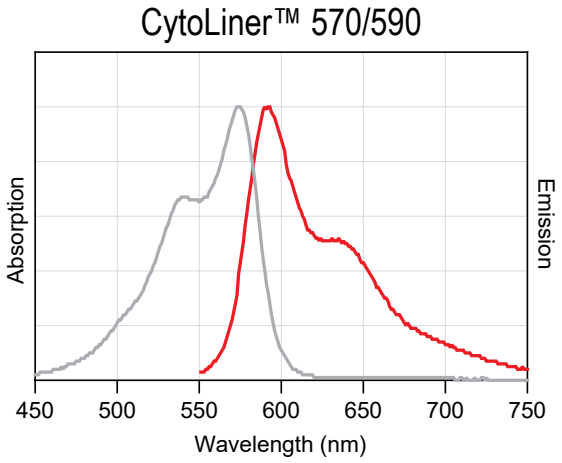

Figure 3. CytoLiner™ 570/590 absorbance and emission spectra.

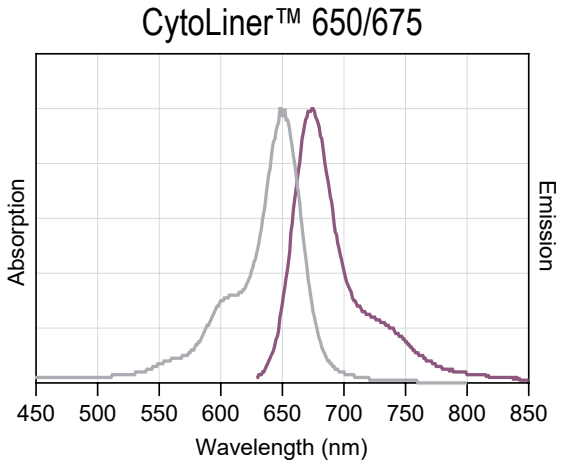

Figure 4. CytoLiner™ 650/675 absorbance and emission spectra.

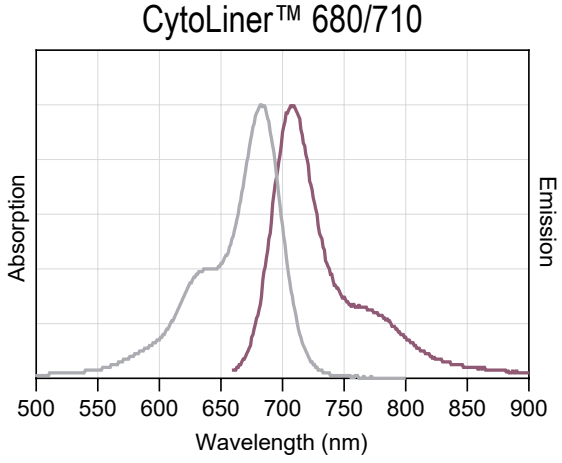

Figure 5. CytoLiner™ 680/710 absorbance and emission spectra.