Product Information

GlycoLiner™ Cell Surface Glycoprotein Labeling Kits

See product page for a full list of product names, unit sizes, and catalog numbers.

Kit Contents

Component	100 labelings*	500 labelings*
GlycoLiner™ Pretreatment Solution, 100X	99898-100UL 100 uL	99898-500UL 500 uL
GlycoLiner™ Reactive Dye or Biotin, 1000X	Component A 20 uL	Component A 100 uL

*Size based on 200 uL labeling volume.

Storage and Handling

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

Product Technical Information

See product page for spectral properties and other dye-specific technical information. See our Spectra Viewer to view and download the dye excitation and emission spectra.

Product Description

GlycoLiner™ Kits are designed for labeling glycoproteins on the surface of live cells with fluorescent dyes or biotin. The reaction is rapid, simple, and gentle, and produces highly selective, covalent labeling of the cell surface that is compatible with subsequent fixation, permeabilization, and immunofluorescence staining or cell lysis. Unlike other covalent cell surface labels, GlycoLiner™ staining of dead cell cytoplasm is less intense than other covalent cell surface labels, which can make imaging of live cell surface staining easier.

The labeling procedure uses aminooxy chemistry to label live cells after a mild oxidation treatment to convert glycoprotein sugars into carbonyl groups (aldehydes and ketones). The reactive GlycoLiner™ Dye or Biotin then reacts with the carbonyl groups to form a stable covalent oxime bond. GlycoLiner™ technology uses novel self-catalyzing aminooxy groups that are highly reactive at neutral pH without the need to add a catalyst. This is in contrast to glycoprotein labeling using conventional aminooxy labels, which is not only slow but also requires the use of high concentrations of reactive dye and catalyst in acidic buffer. The GlycoLiner™ labeling procedure is complete in about 20 minutes, and can be performed at room temperature or 4°C.

Considerations for Staining

- GlycoLiner™ must be used with live cells for cell surface labeling. If cells are fixed first, intracellular structures will be labeled. We recommend using CytoLiner™ Fixed Cell Membrane Stains (see Related Products) for staining formaldehyde-fixed cells.
- GlycoLiner™ may stain FFPE or frozen tissue, but the staining will not be selective for the plasma membrane and will also stain other structures.
- GlycoLiner™ reacts with carbohydrates and aldehyde/ketone groups. Labeling should be done in a buffer like PBS that is free of proteins and sugars. For adherent cells, we recommend using PBS with calcium and magnesium to maintain cell attachment and morphology. Other buffers may be used if they do not contain proteins or carbohydrates.
- GlycoLiner™ is not expected to react with poly-L-lysine-coated surfaces, however, glycosylated matrix components like collagen or proteoglycans in complex matrices like Matrigel® may be labeled.
- Treatment of cells with GlycoLiner™ Pretreatment Solution is required for labeling.
- Labeling at room temperature usually results in highly selective cell surface labeling, however, the procedure also may be performed at 4°C using prechilled buffers at each step to prevent protein internalization during the reaction.
- For cell surface imaging, we recommend imaging or fixing cells shortly after GlycoLiner™ labeling. Labeling is covalent and compatible with commonly used fixation and permeabilization protocols.
- If cells are returned to culture after labeling, the surface labels will become internalized over the course of several hours due to endocytosis of surface glycoproteins. For long-term live-cell surface imaging, we recommend CellBrite® Steady Membrane Staining Kits (see Related Products).
- GlycoLiner™ Dyes and Biotin are cell membrane-impermeant and preferentially react with glycoproteins on the plasma membrane surface. However, GlycoLiner™ also will react with intracellular proteins inside dead cells with compromised plasma membrane integrity. Therefore, GlycoLiner™ labeling cannot be used as the sole criteria for determining whether a labeled protein was localized to the plasma membrane surface at the time of the labeling reaction. Due to the accumulation of glycosylated proteins on the plasma membrane surface, dead cell labeling with GlycoLiner™ tends to be somewhat less intense compared to other reactive cell surface stains, which may make it easier to image surface staining of live cells compared to other stains.

Considerations for Staining (Continued)

- Cells may be lysed after labeling with GlycoLiner™ Biotin to extract biotinylated proteins for downstream capture or analysis using standard procedures.

Experimental Protocols

Protocol overview

1. Rinse live cells with PBS.
2. Remove buffer. Add 1X Pretreatment Solution for 10 minutes.
3. Add an equal volume of 2X GlycoLiner™ Reactive Label to the Pretreatment Solution already on the cells and pipette gently to mix. Incubate for 10 minutes.
4. Rinse cells with PBS. Proceed to imaging, fixation, or lysis.

Staining protocol for adherent cells

The protocol below is based on 200 uL total labeling volume. Scale all volumes proportionally for labeling multiple samples or for labeling in larger culture vessels.

1. Prepare 1X Pretreatment Solution by diluting the 100X stock solution 1:100 in PBS with Ca^{2+}/Mg^{2+}. For example, add 1 uL of 100X Pretreatment Solution to 100 uL of buffer. Prepare enough 1X Pretreatment Solution to completely cover the cells.

   Note: 1X Pretreatment Solution should be prepared fresh shortly before use.
2. Remove the culture medium from live cells and rinse twice with PBS with Ca^{2+}/Mg^{2+}.
3. Remove the buffer and add enough 1X Pretreatment Solution to completely cover the cells. We recommend using 100 uL per well of a 96-well plate.
4. Incubate the cells for 10 minutes at room temperature.
5. Prepare 2X GlycoLiner™ Label by diluting the 1000X stock solution 1:500 in PBS with Ca^{2+}/Mg^{2+}. For example, add 1 uL of 1000X GlycoLiner™ Dye or Biotin to 500 uL of buffer.

   Note: Label concentration may require optimization for specific cell types or applications.
6. Add an equal volume of 2X GlycoLiner™ Label to the Pretreatment Solution already on the cells and immediately pipette gently up and down to mix. For example, if you added 100 uL of 1X Pretreatment Solution per well in step 3, add 100 uL of 2X GlycoLiner™ Label directly to the well for a final concentration of 1X GlycoLiner™ Label in a total volume of 200 uL.
7. Incubate the cells for 10 minutes at room temperature. Cover the plate to protect fluorescent dyes from light.
8. Remove the solution from the cells and rinse twice with PBS with Ca^{2+}/Mg^{2+}.
9. Proceed to fluorescence imaging in the appropriate channel (see product page), or fix cells using your preferred fixation method. Alternatively, if using the GlycoLiner™ Biotin Kit, perform detection with streptavidin or anti-biotin conjugate, or cells may be lysed for extraction and analysis of biotin-labeled proteins.

Staining protocol for cells in suspension

The protocol below is based on 200 uL total labeling volume. Scale all volumes proportionally for labeling multiple samples or for labeling larger cell numbers.

1. Transfer up to one million ($1x10^6$) cells to a microcentrifuge tube.
2. Pellet the cells by centrifuging at 350 x g for 3 minutes.
3. Carefully remove the supernatant and resuspend the pellet in 500 uL of PBS to wash the cells.
4. Repeat steps 2-3 to centrifuge and wash the cells once more.
5. Pellet the cells by centrifuging at 350 x g for 3 minutes.
6. Prepare 1X Pretreatment Solution by diluting the 100X stock solution 1:100 in PBS. For example, add 1 uL of 100X Pretreatment Solution to 100 uL of PBS. Prepare 100 uL of 1x Pretreatment Solution for every million cells.

   Note: 1X Pretreatment Solution should be prepared fresh shortly before use.
7. Carefully remove all traces of the supernatant from the cell pellet and resuspend in 100 uL of 1X Pretreatment Solution.
8. Incubate for 10 minutes at room temperature.
9. Prepare 2X GlycoLiner™ Label by diluting the 1000X stock solution 1:500 in PBS. For example, add 1 uL of 1000X GlycoLiner™ Dye or Biotin to 500 uL of PBS.

   Note: Label concentration may require optimization for specific cell types or applications.
10. Add an equal volume of 2X GlycoLiner™ Label to the Pretreatment Solution already on the cells and immediately pipette gently up and down to mix. For example, if you resuspended one million cells in 100 uL of 1X Pretreatment Solution in step 7, add 100 uL of 2X GlycoLiner™ Label directly to the tube for a final concentration of 1X GlycoLiner™ Label in 200 uL total volume.
11. Incubate the cells for 10 minutes at room temperature. Protect fluorescent dyes from light.
12. Pellet the cells by centrifuging at 350 x g for 3 minutes.
13. Carefully remove the supernatant and resuspend the pellet in 500 uL of PBS to wash the cells.
14. Repeat steps 12-13 to centrifuge and wash the cells once more.
15. Proceed to detection or fixation:
    a. For GlycoLiner™ Dyes: Proceed to fluorescence imaging in the appropriate channel (see product page), or fix cells using your preferred fixation method.
    b. For GlycoLiner™ Biotin: Perform staining and imaging with streptavidin or anti-biotin conjugate using standard procedures, or fix cells using your preferred fixation method for post-fixation biotin detection. Alternatively, cells may be lysed for extraction and analysis of biotin-labeled proteins.

Troubleshooting

Problem	Potential Solutions
No labeling	• Make sure the buffer you are using does not contain sugar or protein. • Check that you correctly diluted the Pretreatment Solution to 1X (1:100). • Make sure to incubate with Pretreatment Solution for 10 minutes before adding the GlycoLiner™ Label. The two steps cannot be done simultaneously. • Pretreatment is not optional; labeling will not occur without it.
Intracellular or nonspecific labeling	• Make sure you are labeling healthy, live cells. If cells are fixed or dead prior to labeling, intracellular targets will be preferentially labeled. • If high background is observed on the culture substrate, check to see if you are using substrate coatings that may contain glycoproteins that could react with aminooxy groups.

Related Products

Cat. No.	Product
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
P033	Biotin Recombinant Monoclonal Mouse Antibody (rBN-34) - Biotium Choice
29030...29129	Streptavidin Conjugates
41033...41040	NucSpot® Nuclear Stains
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
30105...30142	CellBrite® Steady Membrane Staining Kits
30131...30140	CytoLiner™ Fixed Cell Membrane Stains
P023	Nuclear Membrane Recombinant Monoclonal Mouse Antibody (2406.NM) - Biotium Choice
29021...29128	Wheat Germ Agglutinin (WGA) Conjugates
29015...29136	Concanavalin A (Con A) CF® Dye Conjugates
00068...29127	Cholera Toxin Subunit B CF® Dye Conjugates
29060...29137	CF® Dye PNA Lectin *(Arachis hypogaea)*
29012...29132	*Lycopersicon Esculentum* (Tomato) Lectin (LEL, TL) Conjugates
29108...29133	*Ulex Europaeus* Agglutinin I (UEA I) Conjugates
29114...29134	*Phaseolus Vulgaris* Leucoagglutinin (PHA-L) Conjugates
29096...29131	*Datura Stramonium* Lectin (DSL) Conjugates
29120...29135	*Sambucus Nigra* Lectin (SNA, EBL) Conjugates
40081, 40082	NucSpot® Live Cell Nuclear Stains
70058...70086	LysoView™ Dyes
70054...70082	MitoView™ Mitochondrial Dyes
70082	MitoView™ Fix 640
70065, 70069	LipidSpot™ Lipid Droplet Stains
30050...30139	ViaFluor® SE Cell Proliferation Kits
23001, 23002	EverBrite™ Mounting Medium, with or without DAPI
23003...23016	EverBrite™ Hardset Mounting Medium, with or without DAPI or NucSpot® 640
23008, 23009	Drop-n-Stain EverBrite™ Mounting Medium, with or without DAPI

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® Dye antibody conjugates and reactive dyes, bioconjugates, Mix-n-Stain™ antibody labeling kits, and fluorescent stains for visualizing nuclei, mitochondria, or other organelles.

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