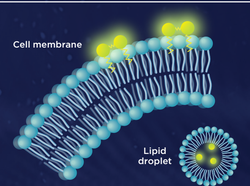
Cellular Stains
Under the Hood

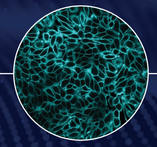
Fluorescent probes, including cell stains and certain protein conjugates, are important tools for examining cellular properties and morphology. Scientists have discovered and developed many different agents for visualizing cells, each designed to identify specific cellular structures or processes. Deciding which probes will work best for a given experiment can be tricky. With this guide, discover how Biotium has optimized cell stains to take advantage of the properties of cellular structures and environments for high specificity and improved signal-to-noise.

LIPOPHILIC MEMBRANE & CELL SURFACE STAINS



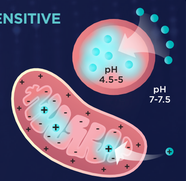
Mammalian cells are enveloped by a membrane with a hydrophobic core that prevents water-soluble molecules and ions from crossing it. Similar membranes surround intracellular organelles such as lipid droplets.

Lipophilic membrane dyes such as **CellBrite® Cytoplasmic Membrane Stains** and **CytoLiner™ Fixed Cell Membrane Dyes** incorporate into cell membranes and are used to visualize the plasma membrane in live or paraformaldehyde-fixed cells. Other lipophilic dyes, such as **LipidSpot™ Lipid Droplet Stains**, are used to visualize cytoplasmic lipid droplets. Lipophilic dyes interact with cellular lipids via hydrophobic interactions and therefore are susceptible to disruption by detergents and organic solvents like glycerol in mounting media.



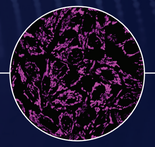
CytoLiner™ 410/450 in fixed Hela cells

ENVIRONMENTALLY-SENSITIVE ORGANELLE STAINS



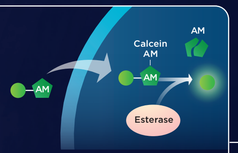
Some organelles such as mitochondria and lysosomes possess unique environmental features that can be used to selectively stain them. Mitochondrial stains target mitochondrial membrane potential generated by oxidative phosphorylation and are used to monitor cell viability and metaboloic activity.
Lysosomal stains target the relatively lower pH environment of the organelle and are helpful for investigating autophagy.

Cationic agents such as **MitoView™ Dyes** are attracted to the negative membrane potential of the mitochondrial matrix. As such, they preferentially migrate to polarized mitochondria. Since the negative membrane potential only exists when the electron transport chain is active, **MitoView™ Dyes**—with the exception of **MitoView™ Green**—are not suitable for fixed cells. **LysoView™ Dyes** are designed to accumulate in low pH organelles such as lysosomes. This type of cell stain is well-suited for live-cell imaging because they are non-toxic and do not require wash steps.



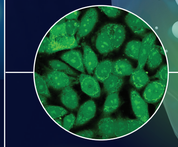
MitoView™ 633 in live HeLa cells

CYTOPLASM STAINS

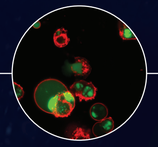


Cytoplasmic staining is useful for visualizing cell counts, cell tracing, monitoring cell proliferation, characterizing cell morphology, and confirming cell viability. For the best results, cytoplasmic dyes should be uniformly distributed throughout the cytoplasm.

ViaFluor® SE Dyes (not shown) are membrane-permeant, amine-reactive fluorogenic molecules that are cleaved by intracellular esterases to create fluorescent dyes. After cleavage, the dyes bind covalently to cytoplasmic amines for fixable staining that can be used to monitor cell division, or track cells in culture. **Calcein AM** is a membrane-permeant dye that is cleaved by intracellular esterases to form green membrane-impermeant fluorescent calcein. Calcein is trapped in the cytoplasm of live cells, but can leak out of dead cells with compromised membranes. Thus, **Calcein AM** may be used to label live cells only, but is not fixable or suitable for long-term cell monitoring.



ViaFluor® 488 SE in live Hela cells

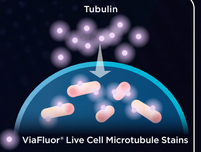


NucView® 488 (green) and CF® 594 Annexin V (red) in apoptotic HeLa cells

NucSpot® Live 650 in live HeLa cells

ViaFluor® 488 Live Cell Microtubule Stain in live HeLa cells

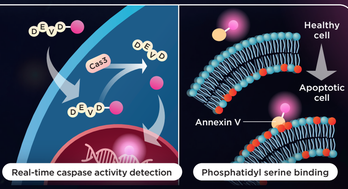
PROTEIN-LIGAND BINDING



Attaching fluorescent dyes to proteins or ligands that bind proteins of interest is a common way to characterize protein expression and identify cellular structures (including the cytoskeleton).

ViaFluor® Live Cell Microtubule Stains are cell-permeant probes for tubulin, suitable for imaging the microtubule cytoskeleton in live cells. **Phalloidin CF® Dye Conjugates** (not shown) may also be used to visualize the cytoskeleton by labeling both small and large F-actin filaments.

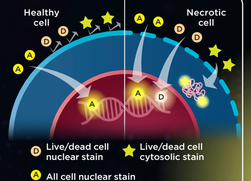
APOPTOSIS REPORTERS



Apoptotic cell death is a biologically important process in life science research. Apoptosis is both multifaceted and well-characterized, meaning that scientists have multiple ways to probe for apoptosis.

Caspase activation is a hallmark of apoptosis, so researchers can use caspase substrates to monitor apoptotic progression. **NucView® Caspase-3 Substrates** are linked to high-affinity DNA-binding dyes. Upon cleavage, the dye is released to bind DNA, at which point it will emit fluorescence. Another effect of apoptosis is the translocation of phosphatidylserine (PS) to the outer leaflet of the plasma membrane. **Annexin V CF® Dye Conjugates** bind to PS, and can therefore be used as an apoptotic cell stain.

DEAD CELL VS. ALL CELL STAINS



Identifying and quantifying dead cells is important for cell culture experiments. Elevated levels of cell death can indicate a problem with the culturing process. Accurate live/dead counts are also important for fields such as pharmacology and molecular biology that often use cell viability/death as an experimental end point.

Necrosis compromises the cell membrane, making it more permeable. Many stains use this as a mechanism to distinguish between healthy and dead or dying cells. **Live-or-Dye™ Fixable Viability Stains**, for example, are only able to cross the membrane of necrotic cells, where they bind covalently to intracellular proteins to label the cytoplasm. Cell-impermeant nuclear-specific dyes such as **NucSpot® Nuclear Stains** and **RedDot™ 2** work similarly. However, upon crossing the cell membrane in necrotic tissues, the dyes localize and bind to DNA in the nucleus. Alternatively, "all cell" stains such as **NucSpot® Live** and **Hoechst dyes** can cross healthy cell membranes to stain the nucleus. To identify live and dead cells in the same sample, researchers use both all cell stains and dead cell stains.

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