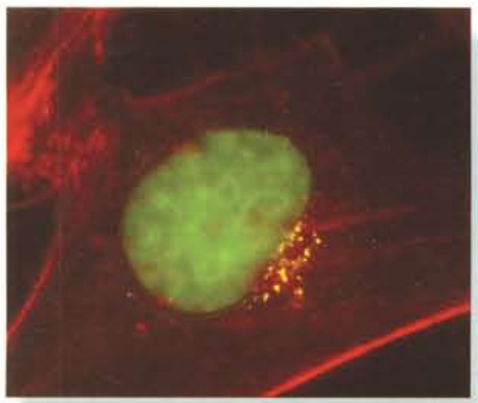
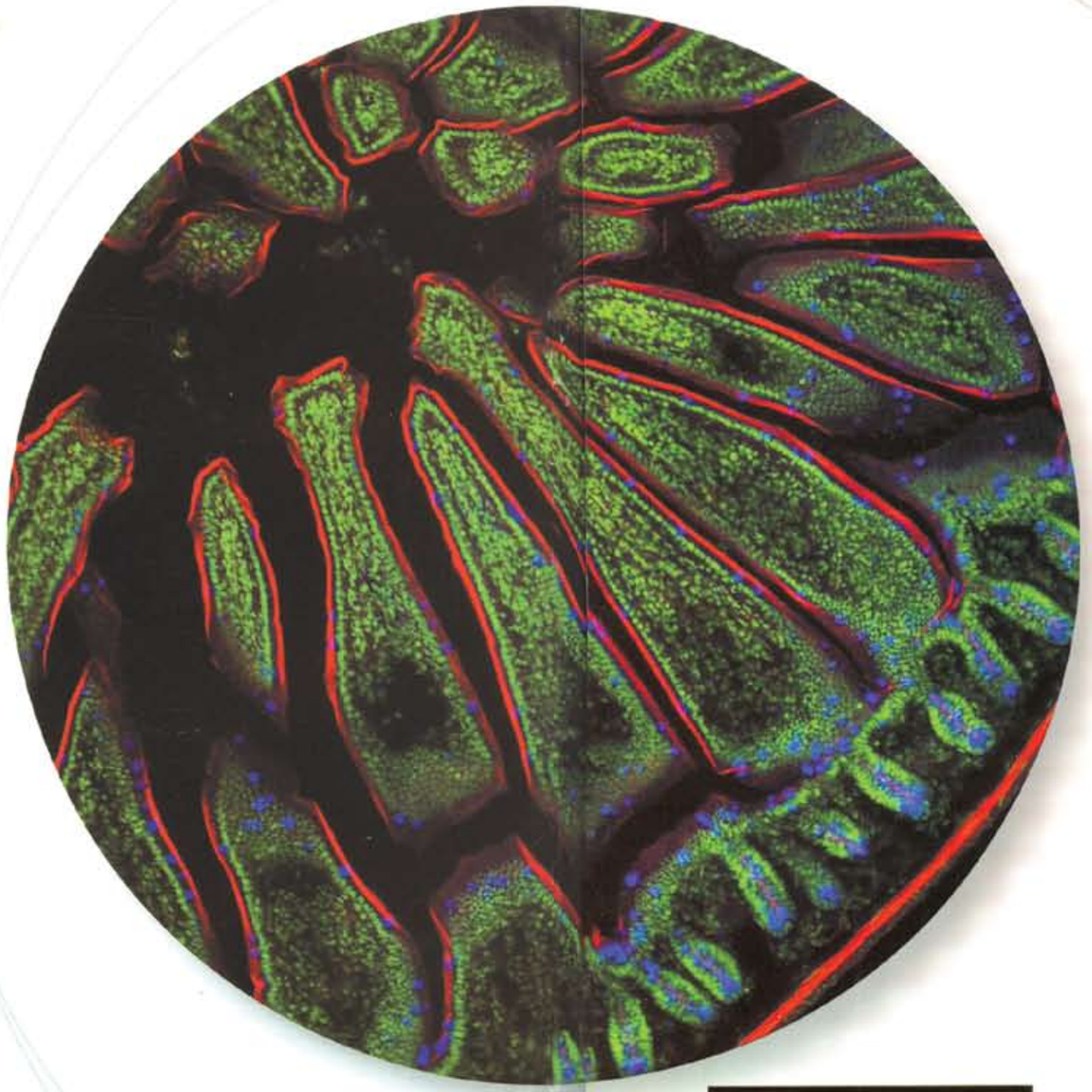
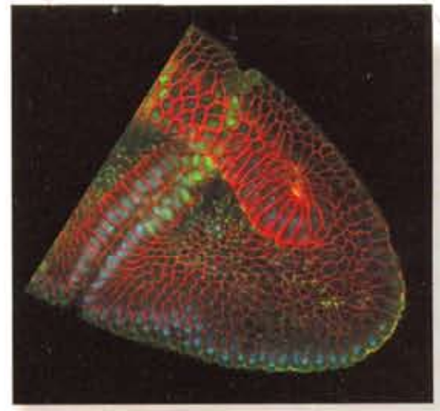
BioProbes 32

December 1999







New Products and Applications

Ultrasensitive Protein Staining without Fixatives

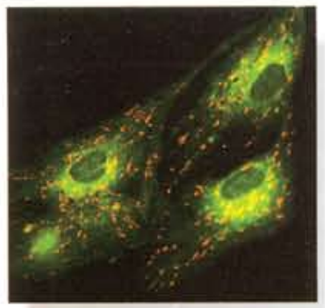
New SYPRO Tangerine protein gel stain

Streamline Your IEF Gel Processing

New SYPRO Ruby IEF protein gel stain

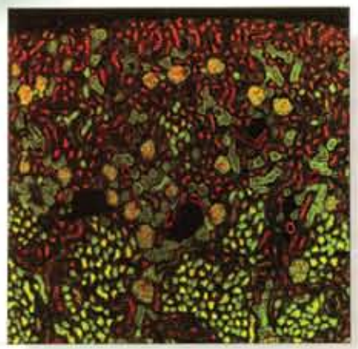
High-Performance Calcium Indicators

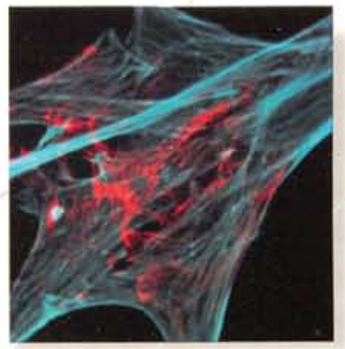
- Fluo-3 for high-throughput screening
- Lower-affinity analogs of fluo-4
- Five new fura analogs
- Ultrapure fura-2 AM



Tools for Cell Biology Research

- Membrane permeability assays
- Cell proliferation assays
- Golgi-specific probes





Molecular Probes, Inc.

PO Box 22010
Eugene, OR 97402-0469
Phone: (541) 465-8300
Fax: (541) 344-6504

Customer Service
Phone: (541) 465-8338
Fax: (541) 344-6504
E-mail: order@probes.com

For US and Canada
Toll-Free Order Phone: (800) 438-2209
Toll-Free Order Fax: (800) 438-0228

Technical Assistance
Phone: (541) 465-8353
Fax: (541) 465-4593
E-mail: tech@probes.com

Molecular Probes Europe BV

PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378
Fax: +31-71-5233419

Customer Service
Phone: +31-71-5236850
Fax: +31-71-5233419
E-mail: eurorder@probes.nl

Technical Assistance
Phone: +31-71-5233431
Fax: +31-71-5233419
E-mail: eurotech@probes.nl

Visit Molecular Probes at Our Web Site
www.probes.com

The *Handbook, 7th Edition* is now available on CD-ROM.

The Leader in Fluorescence Technology

US32-99

Molecular Probes

Membrane Permeability Analysis

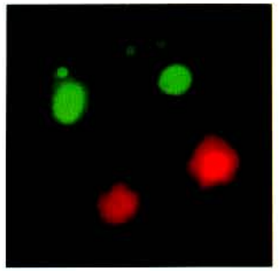


Figure 15. Apoptosis induced in Jurkat cells with 10 µM camptothecin. The cells were then treated with the reagents in the Vybrant Apoptosis Assay Kit #4. Early apoptotic cell nuclei were labeled with YO-PRO-1 dye (green). Late stage apoptotic and necrotic cells were detected with propidium iodide (red).

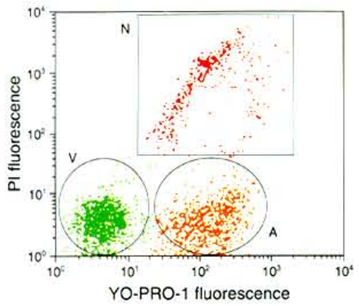


Figure 16. Jurkat cells (T-cell leukemia, human) exposed to 10 µM camptothecin for four hours. Cells were then treated with the reagents in the Vybrant Apoptosis Assay Kit #4 followed by flow cytometric analysis. V = viable cells, N = necrotic cells, A = apoptotic cells.

References

1. J Biol Chem 273, 18122 (1998); **2.** Science 272, 735 (1996); **3.** J Biol Chem 272, 5482 (1997); **4.** Neuropharmacology 36, 1285 (1997); **5.** J Physiol 519, 335 (1999); **6.** J Immunol Meth 185, 249 (1995).

When the intact plasma membrane of a live cell prevents cationic nucleic acid stains such as propidium iodide (P-1304) and SYTOX® Green stain (S-7020) from interacting with nuclear DNA, the detected fluorescence signal is inversely proportional to the integrity of the membrane, and also therefore to cellular viability. Researchers have developed refinements of this widely used technique, three of which are reviewed here.

Pore Size Analysis

Dyes of different sizes can be used to probe the dimensions of membrane pores. Membrane depolarization caused by the lytic bacterial toxin aerolysin has been correlated[1] with permeability of **ethidium bromide** (E-1305) but not **ethidium homodimer-1** (E-1169). The selective entry of the smaller dye indicates that depolarization is due to small pores formed by the toxin and not by loss of overall membrane integrity. This result also implies that the larger homodimer nucleic acid stains may be more stringent indicators of cell viability than the smaller monomers. Another pair of dyes that may be useful for this purpose is **YO-PRO®-1 dye** (Y-3603) and **YOYO®-1 dye** (Y-3601). YOYO-1 is twice the size of YO-PRO-1 dye but has very similar spectral properties.

Detecting Expression and Activation of P2X Receptors

The $P2X_7$ receptor is a member of the P2X family of ATP-activated ion channels. Activation of the macrophage $P2X_7$ receptor induces the opening of pores that are permeable to large cations and, if sustained, eventually results in cell lysis.[2] $P2X_7$ receptor activation can be detected by measuring the fluorescence increase resulting from passage of extracellular **YO-PRO-1 dye** (Y-3603) through the cation pore. Researchers have used this assay to make functional comparisons between rat and human $P2X_7$ receptors,[3] to analyze the activation-modulating effects of divalent cations[4] and to determine that the initial dilation of the cation pore is reversible.[5] Neuronal P2X receptors also exhibit progressive pore dilation that is detectable by YO-PRO-1 dye uptake.[5] In this case, pore dilation does not proceed to cell lysis; instead, it appears that the induced permeability changes may be an important mechanism for modulating synaptic transmission.

Detection of Apoptotic Cells

The membranes of apoptotic cells, although intact, become permeable to certain fluorescent dyes including YO-PRO-1 dye (Y-3603) but not to propidium iodide. Selective uptake of YO-PRO-1 dye by apoptotic cells has been demonstrated in three different cell types.[6] Propidium iodide penetrates only necrotic cells where it displaces YO-PRO-1 dye, allowing necrotic and apoptotic cells to be clearly discriminated by either fluorescence microscopy (Figure 15) or flow cytometry (Figure 16). Our **Vybrant™ Apoptosis Assay Kit #4** (V-13243) provides ready-to-use solutions of both YO-PRO-1 dye and propidium iodide, and an optimized protocol.

Cat #	Product Name	Unit Size	Price Per Unit ($) 1–4 Units	5–24 Units
E-1305	ethidium bromide	1 g	16.00	12.80
E-1169	ethidium homodimer-1 (EthD-1)	1 mg	178.00	142.40
P-1304	propidium iodide	100 mg	60.00	48.00
S-7020	SYTOX® Green nucleic acid stain *5 mM solution in DMSO*	250 µL	125.00	100.00
V-13243	Vybrant™ Apoptosis Assay Kit #4 *YO-PRO®-1/propidium iodide* *200 assays*	1 kit	125.00	100.00
Y-3603	YO-PRO®-1 iodide (491/509) *1 mM solution in DMSO*	1 mL	158.00	126.40
Y-3601	YOYO®-1 iodide (491/509) *1 mM solution in DMSO*	200 µL	265.00	212.00

Golgi-Specific Probes for Live and Fixed Cells

New Anti-Golgi Antibody

Molecular Probes is pleased to offer researchers the **anti–human golgin-97, mouse monoclonal CDF4 antibody**[1] (A-21270, Figure 17). Golgin-97, a member of the granin family of proteins, is an integral membrane protein localized on the cytoplasmic face of the Golgi complex.[2,3] This new antibody should be useful for immunohistochemical and immunofluorescent detection and identification of the Golgi complex.

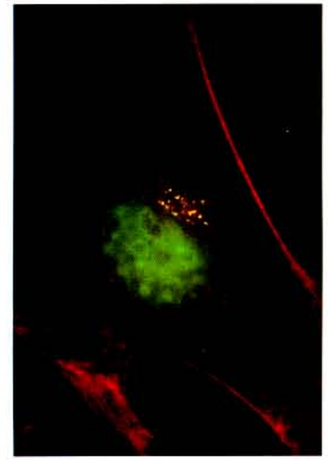


Figure 17. HeLa cells were fixed and permeabilized, then treated with RNase. The cells were labeled with anti–human golgin-97 antibody, then stained with Alexa Fluor 430 goat anti–mouse IgG (H+L) antibody (A-11063). The cells were then labeled with Alexa Fluor 594 phalloidin (A-12381) and finally, with SYTOX® Green nucleic acid stain (S-7020).

Monitor Golgi in Live Cells

The **NBD** and **BODIPY® FL conjugates of ceramide** (N-1154, D-3521) and their **sphingomyelin** metabolites (N-3524, D-3522) are effective probes for the flattened membranous sacs of the Golgi complex in live cells.[4] Researchers have used these conjugates to study lipid transport pathways,[5-7] visualize cellular morphology,[8-10] and measure metabolite formation and subsequent localization.[11,12]

Although both of these fluorophores can be visualized with standard fluorescein optics, researchers have been able to exploit the unique spectral properties of each fluorochrome for specific applications. For example, at high concentrations **BODIPY FL C_5-ceramide** (D-3521) forms excimers, resulting in a shift of the fluorophore's emission maximum (Figure 18) from 515 nm (green) to ~620 nm (red). This property can be used to quantitate BODIPY FL C_5-ceramide accumulation using ratiometric imaging.[13,14]

Alternatively, the photobleaching rate of **NBD C_6-ceramide** (N-1154) appears to be sensitive to the cholesterol content of the Golgi apparatus, a phenomenon that is not observed with BODIPY FL C_5-ceramide.[15] Because they localize to endogenous lipids and cholesterol, these fluorescent conjugates can also be used to stain fixed cells.[16]

Complexing fluorescent ceramides with serum albumin (BSA) facilitates cell labeling without requiring the use of organic solvents to dissolve the probe.[17] For this application, we now also provide **BODIPY FL C_5-ceramide** and **NBD ceramide complexed with defatted BSA** (B-22650, N-22651).

Stain Golgi in Fixed Cells

As an alternative to using standard immunohistochemical techniques, researchers can use the specificity of wheat germ agglutinin (WGA) to *N*-acetylglucosamine to visualize the Golgi complex in aldehyde-fixed and permeabilized cells.[18] Below is a simplified protocol:

- Fix cells with an aldehyde-based fixative (3% formaldehyde and 0.1% glutaraldehyde at room temperature for ten minutes)
- Permeabilize cells with cold methanol at -20 °C
- Wash with buffer and block with 1% BSA
- Incubate with fluorescent WGA (~200 ng/mL) for 30 minutes at room temperature[19]
- Wash, mount and visualize using appropriate filter sets

Table 5 lists our complete selection of **fluorescent WGA conjugates**. If you would like to test a variety of fluorescent WGA conjugates before you choose one, we also offer a **Wheat Germ Agglutinin Sampler Kit** (W-7024), which contains 1 mg quantities each of WGA conjugated to Alexa Fluor™ 350, Oregon Green® 488, tetramethylrhodamine and Texas Red®-X dyes.

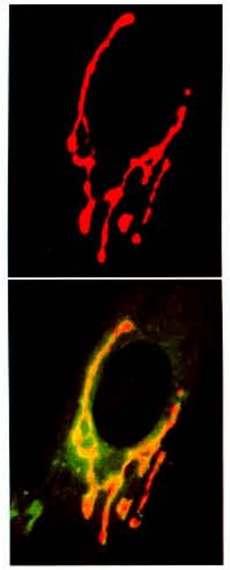


Figure 18. Selective staining of the Golgi apparatus using BODIPY FL C_5-ceramide. Images contributed by Richard Pagano, Mayo Foundation.