15.2 Viability and Cytotoxicity Assay Reagents

Fluorometric assays of cell viability and cytotoxicity are easy to perform with the use of a fluorescence microscope, fluorometer, fluorescence microplate reader or flow cytometer,[1] and they offer many advantages over traditional colorimetric and radioactivity-based assays. This section describes our numerous reagents for conducting viability and cytotoxicity assays in a wide variety of cells, including those of animal origin as well as bacteria and yeast. Following this discussion of individual reagents is Section 15.3, which contains a thorough description of each of our viability and cytotoxicity kits, including the:

- LIVE/DEAD Viability/Cytotoxicity Kit (L-3224)
- LIVE/DEAD Reduced Biohazard Cell Viability Kits (L-7013, L-23101, L-23102, L-23105)
- LIVE/DEAD Cell-Mediated Cytotoxicity Kit (L-7010)
- Vybrant Tumor Necrosis Factor (TNF) Assay Kit (V-23100)
- LIVE/DEAD Sperm Viability Kit (L-7011)
- LIVE/DEAD Yeast Viability Kit (L-7009)
- LIVE/DEAD *Bac*Light Bacterial Viability Kits (L-7007, L-7012, L-13152)
- ViaGram Red$^+$ Bacterial Gram Stain and Viability Kit (V-7023)
- Vybrant Cytotoxicity Assay Kit (V-23111)
- Vybrant Cell Metabolic Assay Kit with C_{12}-resazurin (V-23110)

Many of these reagents and assay kits have been developed and patented by Molecular Probes' scientists and are exclusively available from Molecular Probes and its distributors. Also discussed in this section and Section 15.3 are our unique single-step reagents and kits for assessing gram sign and for simultaneously determining gram sign and viability of bacteria, as well as our novel fluorescent antibiotics. Section 15.4 describes our important probes for quantitating cell proliferation, analyzing the cell cycle and detecting the presence of bacteria and mycoplasma.

Viability/Cytotoxicity Assays Using Esterase Substrates

Molecular Probes prepares a wide variety of fluorogenic esterase substrates — including calcein AM, BCECF AM and various fluorescein diacetate derivatives — that can be passively loaded into adherent and nonadherent cells. These cell-permeant esterase substrates serve as viability probes that measure both enzymatic activity, which is required to activate their fluorescence, and cell-membrane integrity, which is required for intracellular retention of their fluorescent products.

As electrically neutral or near-neutral molecules, the esterase substrates freely diffuse into most cells. In general, cell loading of acetate or acetoxymethyl ester derivatives is accomplished by initially preparing a 1–10 mM stock solution of the dye in dimethylsulfoxide (DMSO) and then diluting the stock solution into the cell medium to a final concentration of 1–25 μM (see Loading and Calibration of Intracellular Ion Indicators in Section 20.1). Once inside the cell, these nonfluorescent substrates are converted by nonspecific intracellular esterases into fluorescent products that are retained by cells with intact plasma membranes. In contrast, both the unhydrolyzed substrates and their products rapidly leak from dead or damaged cells with compromised membranes, even when the cells retain some residual esterase activity. Low incubation temperatures and highly charged esterase products usually favor retention, although the rate of dye loss from viable cells also depends to a large extent on cell type (see Multidrug Resistance in Section 15.6). For example, mast cells and epithelial cells actively secrete many polar products.[2,3] Table 15.1 lists Molecular Probes' esterase substrates that have been used for cell viability studies and compares their cell loading, retention and pH sensitivity. Many of the applications of these esterase substrates — for example, viability, cytotoxicity and adhesion assays — closely parallel those of ^{51}Cr-release assays, except that the fluorescent probes do not carry the risks or the disposal costs associated with the use of radioactive materials.

Calcein AM, Dihydrocalcein AM and Calcein Blue AM

Of the dyes listed in Table 15.1, calcein AM (C-1430, C-3099, C-3100; Figure 15.2) stands out as the premier indicator of cell viability due to its superior cell retention and the relative insensitivity of its fluorescence to pH in the physiological range.[4–7] Calcein AM is also the best single probe available for use in assays for cell adhesion, chemotaxis and multidrug resistance (Section 15.6). Calcein (C-481, Section 14.3), which is the hydrolysis product of calcein AM, is a polyanionic fluorescein derivative (Figure 14.32) that has about six negative charges and two positive charges at pH 7.[8] Calcein is better retained in viable cells than are fluorescein, carboxyfluorescein and BCECF (Figure 15.3) and tends to have brighter fluorescence in a number of mammalian cell types. Calcein AM has the ability to penetrate intact cornea, revealing cell viability, morphology and organization of living cornea.[9,10] Furthermore, unlike some other dyes — including BCECF AM — calcein AM does not interfere with leukocyte chemotaxis or superoxide production, nor does it affect lymphocyte–target cell conjugation.[6,11–14] Leakage of calcein from calcein AM–loaded cells has been used to measure the increase in membrane permeability that occurs above physiological temperatures,[15] as well as to assay for cytotoxic T lymphocyte activity.[5] Fluorescence of extracellular calcein that has leaked from cells or that has been lost during secretion, lysis or ATP-dependent anion transport can be quenched by 5 μM Co^{2+} ion or by Mn^{2+} ion. Heavy-atom quenching of calcein provides a means of detecting dye leakage and quantitating only the intracellular fluorescence.[16]

Dihydrocalcein AM (Figure 15.4) is a reasonably stable, chemically reduced form of calcein AM that requires *both* hydrolysis by intracellular esterases and oxidation within the cell to produce the green-fluorescent calcein dye. Dihydrocalcein AM resembles 2′,7′-dichlorodihydrofluorescein diacetate (D-399, Figure 16.1), the important indicator for oxidative activity in cells (see below), except that its oxidation product (calcein, Figure 14.32) should be better retained in cells than is the oxidation product of 2′,7′-dichlorodihydrofluorescein diacetate. Dihydrocalcein AM (D-23805) is available as a set of 20 vials, each containing 50 μg of the product.

We also offer calcein blue AM (C-1429, Figure 15.5), a viability indicator for use with instruments optimized for the detection of blue fluorescence.[17,18] Calcein blue AM is useful for viability measurements in combination with our SYTOX Green nucleic acid stain (see below) and other green- or red-fluorescent probes.

BCECF AM

BCECF AM (B-1150, B-1170, B-3051) is extensively used for detecting cytotoxicity [19] and for determining the ability of surviving cells to proliferate.[20,21] The intracellular hydrolysis product of BCECF AM, BCECF (B-1151, Section 21.2), has 4–5 negative charges (Figure 21.6), a property that considerably improves its cell retention in viable cells over that of fluorescein or carboxyfluorescein (Figure 15.3). However, because the emission intensity of BCECF is only half-maximal at pH 7.0 (pK_a = 6.98, Figure 21.3) — and is even further reduced in a cell's acidic compartments — the signal intensity of BCECF may be less than optimal in some cell viability and cell adhesion assays.

Using monoclonal antibodies known to either enhance or inhibit natural killer (NK) cell function, researchers found that BCECF AM was at least as effective as ^{51}Cr for measuring NK activity. Furthermore, the fluorescence-based assay could be performed with smaller samples.[22] BCECF AM has also been used to screen for trypanocidal activity [23] and viability of islets.[24]

Table 15.1 Esterase substrates for cell viability studies.

Esterase Substrate (Cat #)	Properties in Cells	pK_a of Product *
BCECF AM (B-1150, B-1170, B-3051)	• Quite well retained • Released during cytolysis • pH-sensitive fluorescence	7.0
Calcein AM (C-1430, C-3099, C-3100)	• Quite well retained • Released during cytolysis • Not as pH-sensitive as BCECF	5
Carboxyeosin diacetate, succinimidyl ester (C-22803, Section 15.4)	• Well retained by reaction with amines • Useful for DAB photoconversion • Phosphorescent	<5 †
Carboxy-2′,7′-dichlorofluorescein diacetate (carboxy-DCFDA, C-369)	• Moderately well retained • Not as pH-sensitive as CFDA	4.8
Carboxyfluorescein diacetate (5(6)-CFDA, C-195)	• Moderately well retained • pH-sensitive fluorescence	6.4
Carboxyfluorescein diacetate, acetoxymethyl ester (5-CFDA, AM; C-1354)	• Easier to load than CFDA yet yields the same product upon hydrolysis • pH-sensitive fluorescence	6.4
Carboxyfluorescein diacetate, succinimidyl ester (5(6)-CFDA, SE; C-1157, Section 15.4)	• Well retained by reaction with amines • Not completely released during cytolysis • pH-sensitive fluorescence	6.4 †
CellTracker Green CMFDA (CMFDA; C-2925, C-7025)	• Well retained by reaction with thiols • Not completely released during cytolysis • pH-sensitive fluorescence	6.4 †
Chloromethyl SNARF-1, acetate (C-6826)	• Well retained by reaction with thiols • Not completely released during cytolysis • Long-wavelength, pH-sensitive fluorescence	7.5 †
Fluorescein diacetate (F-1303)	• Poorly retained • pH-sensitive fluorescence • Inexpensive	6.4
Oregon Green 488 carboxylic acid diacetate (carboxy-F_2FDA, O-6151)	• Moderately well retained • Not as pH-sensitive as CFDA	4.7

* Approximate pK_a values in aqueous solvents. The actual pK_a of the indicator will vary somewhat depending upon experimental conditions. † pK_a of the unconjugated hydrolysis product; after conjugation to an intracellular amine or thiol, the actual pK_a value may be different.

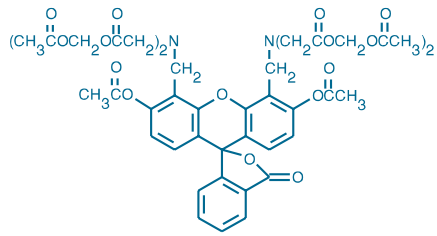

Figure 15.2 C-1430 calcein, AM.

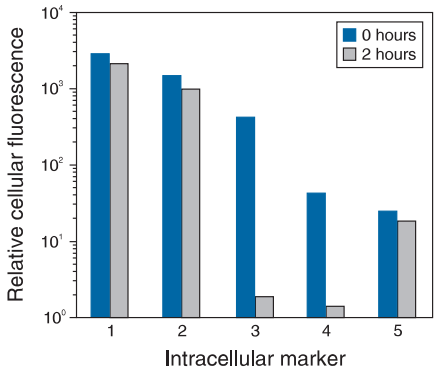

Figure 15.3 Loading and retention characteristics of intracellular marker dyes. Cells of a human lymphoid line (GePa) were loaded with the following cell-permeant acetoxymethyl ester (AM) or acetate derivatives of fluorescein: 1) calcein AM (C-1430, C-3099, C-3100), 2) BCECF AM (B-1150), 3) fluorescein diacetate (FDA, F-1303), 4) carboxyfluorescein diacetate (CFDA) (C-1354) and 5) CellTracker Green CMFDA (5-chloromethylfluorescein diacetate, C-2925, C-7025). Cells were incubated in 4 μM staining solutions in Dulbecco's modified eagle medium containing 10% fetal bovine serum (DMEM+) at 37°C. After incubation for 30 minutes, cell samples were immediately analyzed by flow cytometry to determine the average fluorescence per cell at time zero (0 hours). Retained cell samples were subsequently washed twice by centrifugation, resuspended in DMEM+, maintained at 37°C for 2 hours and then analyzed by flow cytometry. The decrease in the average fluorescence intensity per cell in these samples relative to the time zero samples indicates the extent of intracellular dye leakage during the 2-hour incubation period.

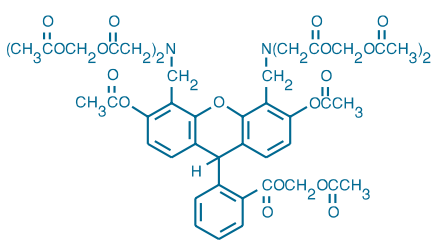

Figure 15.4 D-23805 dihydrocalcein, AM.

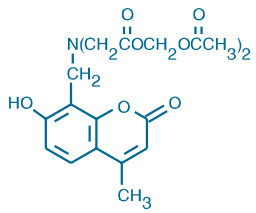

Figure 15.5 C-1429 calcein blue, AM.

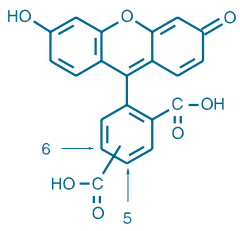

Figure 15.6 C-194 5-(and-6)-carboxyfluorescein.

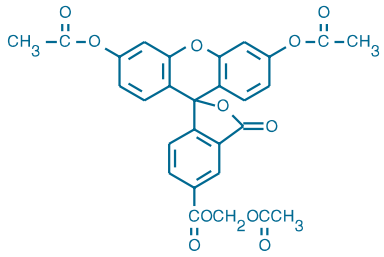

Figure 15.7 C-1354 5-carboxyfluorescein diacetate, acetoxymethyl ester.

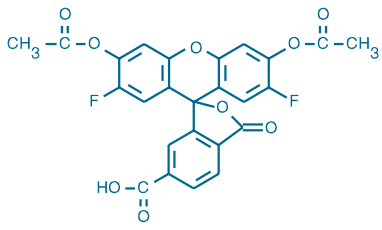

Figure 15.8 O-6151 Oregon Green 488 carboxylic acid diacetate.

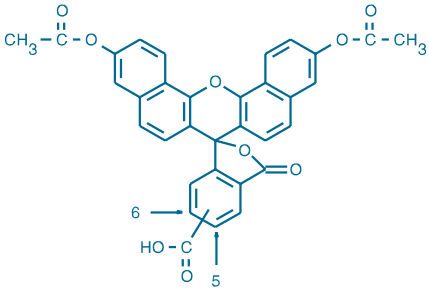

Figure 15.9 C-13196 5-(and-6)-carboxynaphthofluorescein diacetate.

Fluorescein Diacetate

Fluorescein diacetate (FDA, F-1303) was one of the first probes to be used as a fluorescent indicator of cell viability.[25–27] FDA is still occasionally used to detect cell adhesion [28] or, in combination with propidium iodide (P-1304; P-3566; FluoroPure grade, P-21493), to determine cell viability.[29,30] However, fluorescein, (F-1300, Section 21.2) which is formed by intracellular hydrolysis of FDA, rapidly leaks from cells (Figure 15.3). Thus, other cell-permeant dyes such calcein AM and BCECF AM are now preferred for cell viability assays.

Carboxyfluorescein Diacetate and Its Derivatives

The high leakage rate of fluorescein from cells [27,31] prompted the development of carboxyfluorescein diacetate (CFDA), which was originally used to measure intracellular pH [32] but was soon adapted for use as a cell viability indicator.[33,34] Upon hydrolysis by intracellular nonspecific esterases, CFDA forms carboxyfluorescein (5(6)-FAM, C-194, C-1904; Section 14.3). As compared to fluorescein, carboxyfluorescein contains extra negative charges (Figure 15.6) and is therefore better retained in cells [6] (Figure 15.3). CFDA is moderately permeant to most cell membranes, with uptake greater at pH 6.2 than at pH 7.4.[32] The mixed-isomer preparation of CFDA (5(6)-CFDA, C-195) is usually adequate for cell viability measurements; however, we also prepare high-purity single isomers of CFDA (C-1361, C-1362). In addition, we offer the electrically neutral AM ester of CFDA (5-CFDA, AM; C-1354; Figure 15.7), which can be loaded into cells at lower concentrations than CFDA. Upon hydrolysis by intracellular esterases, this AM ester also yields carboxyfluorescein.[35–37] CFDA, CFDA AM and sulfofluorescein diacetate (see below) have been proposed for detection of living organisms on Mars.[38] Hemoglobin can be used to quench extracellular fluorescence due to leakage of probes or leakage of products, such as fluorescein or carboxyfluorescein.[39] Alternatively, antibodies directed against the fluorescein hapten (Section 7.4, Table 7.13) or the membrane-impermeant reagent trypan blue can be used to quench low levels of extracellular fluorescence of some fluorescein-based dyes (Figure 16.23).

CFDA has been used as a viability probe with a variety of cells, including bacteria,[40] fungi (e.g., *Saccharomyces cerevisiae*),[41] spermatozoa,[42] natural killer (NK) cells [19,43] and tumor cells.[44] Cytotoxicity assays using either CFDA or 5-(and-6)-carboxy-2′,7′-dichlorofluorescein diacetate (carboxy-DCFDA, C-369) show good correlation with results obtained using the radioisotopic ^{51}Cr-release method.[19,45] With its low pK_a, carboxy-DCFDA is frequently used as a selective probe for the relatively acidic yeast vacuole.[46–48] Oregon Green 488 carboxylic acid diacetate (carboxy-DFFDA, O-6151; Figure 15.8) also exhibits a low pK_a (~4.7, Figure 21.31) and may be similarly useful as a vital stain. Its intracellular hydrolysis product — Oregon Green 488 carboxylic acid (O-6146, Section 1.5, Figure 1.58) — is more photostable than carboxyfluorescein (Figure 1.42).

Sulfofluorescein Diacetate

Sulfofluorescein diacetate (SFDA, S-1129), which is converted by intracellular esterases to fluorescein sulfonic acid (F-1130, Section 14.3), is more polar than CFDA and consequently may be more difficult to load into some viable cells. However, SFDA's polar hydrolysis product, fluorescein sulfonic acid, is better retained in viable cells than is carboxyfluorescein.[49–52] SFDA was used to stain live bacteria and fungi in soil; little interference from autofluorescence of soil minerals or detritus was observed.[53]

CellTracker Green CMFDA

Molecular Probes' patented CellTracker dyes are thiol-reactive fluorescent dyes that are retained in many live cells through several generations and are not transferred to adjacent cells in a population (Figure 14.5, Figure 14.6, Figure 14.15), except possibly through gap junctions. These dyes represent a significant breakthrough in the cellular retention of fluorescent probes and are ideal long-term tracers for transplanted cells or tissues (Section 14.2).

CellTracker Green CMFDA (C-2925, C-7025) freely diffuses into the cell, where its weakly thiol-reactive chloromethyl moieties react with intracellular thiols and their acetate groups are cleaved by cytoplasmic esterases (Figure 14.7), generating the fluorescent product (Figure 14.4). The other CellTracker probes (coumarin, BODIPY and tetramethyl-

rhodamine derivatives; Section 14.2) do not require enzymatic cleavage to activate their fluorescence. Because the CellTracker dyes may react with both glutathione and proteins, cells with membranes that become compromised after staining may retain some residual fluorescent conjugates. However, use of a membrane-impermeant probe such as propidium iodide (P-1304; P-3566; FluoroPure grade, P-21493), SYTOX Blue (S-11348), SYTOX Orange (S-11368) or one of our "dimeric" or "monomeric" nucleic acid stains (see below) in combination with CellTracker Green CMFDA should permit relatively long-term cytotoxicity assays. CellTracker Green CMFDA and ethidium homodimer-1 (EthD-1, E-1169) have been used to detect viable and nonviable cells in rat and human coronary and internal thoracic arteries sampled at autopsy [54] and in connective tissue explants.[55]

Pentafluorobenzoyl Aminofluorescein Diacetate

The green-fluorescent 5-(pentafluorobenzoyl)aminofluorescein (PFB-F, P-12925; Section 14.3) — the intracellular hydrolysis product of PFB aminofluorescein diacetate (PFB-FDA, P-12880) — localizes in the cytoplasm, is well-retained in cells with intact cell membranes (even through cell division) and is fixable by formaldehyde and glutaraldehyde. PFB-F appears to be retained in viable cells by two mechanisms: 1) retention of the relatively lipophilic PFB group of the hydrolysis products in the cell membrane, and 2) glutathione *S*-transferase–catalyzed reaction of the hydrolysis products with intracellular glutathione.[56] In addition, the relatively high lipophilicity of PFB-FDA facilitates passive cell loading: cells are simply incubated at 37°C in 1–10 µM PFB-FDA in standard culture medium. Thus, like our CellTracker Green CMFDA, PFB-FDA should prove useful for long-term cell labeling.

Chloromethyl SNARF-1 Acetate

Chloromethyl SNARF-1 acetate [57] (C-6826, Figure 14.17) is the only cell-tracking dye (and pH indicator) that exhibits bright-red cytoplasmic fluorescence (Figure 14.19) when excited at the same wavelengths used to excite the green-fluorescent hydrolysis product of CMFDA (Figure 21.11). The spectral characteristics of these two dyes permit simultaneous tracking of two cell populations by either fluorescence microscopy or flow cytometry. The large Stokes shift of the SNARF fluorophore also makes chloromethyl SNARF-1 acetate useful as a viability indicator in cells that exhibit green autofluorescence when excited by the 488 nm spectral line of the argon-ion laser.

Carboxynaphthofluorescein Diacetate

Carboxynaphthofluorescein diacetate (C-13196, Figure 15.9), which is cleaved by intracellular esterases to yield red-fluorescent carboxynaphthofluorescein (excitation/emission maxima ~598/668 nm), is the only long-wavelength tracer of this type that can be passively loaded into live cells.[58] Like chloromethyl SNARF-1 acetate, carboxynaphthofluorescein diacetate is usually used in combination with a green-fluorescent tracer for detecting cell–cell interactions.

Viability/Cytotoxicity and Gram Stain Assays Using Nucleic Acid Stains

To simultaneously detect both the live-cell and dead-cell populations, viability assessments of animal cells, bacteria and yeast frequently employ polar and therefore cell-impermeant nucleic acid stains to detect the dead-cell population. Nucleic acid stains are most often used in combination with intracellular esterase substrates (see above), membrane-permeant nucleic acid stains (see below), membrane potential–sensitive probes (Chapter 23), organelle probes (Chapter 12) or cell-permeant indicators (Chapter 20, Chapter 21 and Chapter 22) to detect the live-cell population. Although many other cell-impermeant dyes could be used to detect dead cells, the high concentrations of nucleic acids in cells, coupled with the large fluorescence enhancement exhibited by most of our nucleic acid stains upon binding, make cell-impermeant nucleic acid stains the logical candidates for viability probes. See Table 8.2 for a list of several cell-impermeant nucleic acid stains and Section 8.1 for a general discussion of dye binding to nucleic acids.

SYTOX Green, SYTOX Orange and SYTOX Blue Nucleic Acid Stains

Many polar nucleic acid stains will enter eukaryotic cells with damaged plasma membranes yet will not stain dead bacteria with damaged plasma membranes. Our patented SYTOX Green nucleic acid stain [59] (S-7020) is a high-affinity probe that easily penetrates eukaryotic cells and both gram-positive and gram-negative bacteria with compromised plasma membranes, yet is completely excluded from live cells.[60] After brief incubation with the SYTOX Green nucleic acid stain, dead bacteria fluoresce bright green when excited with the 488 nm spectral line of the argon-ion laser or any other 470–490 nm source (Figure 15.10). These properties, combined with its ~1000-fold fluorescence enhancement upon nucleic acid binding, make our SYTOX Green stain a simple and quantitative dead-cell indicator for use with fluorescence microscopes, fluorometers, fluorescence microplate readers or flow cytometers (Figure 15.11). We have taken advantage of the sensitivity of the SYTOX Green nucleic acid stain in our ViaGram Red$^+$ Bacterial Gram Stain and Viability Kit (V-7023) and in our Vybrant Apoptosis Assay Kit #1 (V-13240, Section 15.5). A significant application of the SYTOX Green nucleic acid

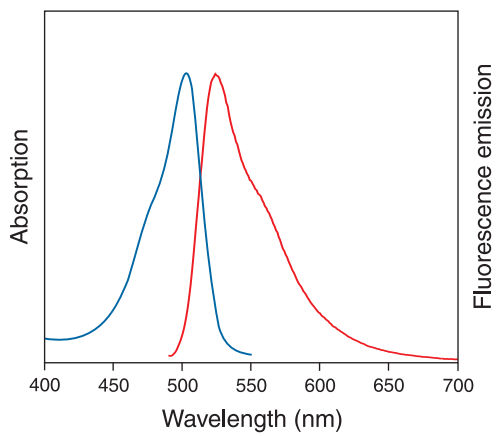

Figure 15.10 Absorption and fluorescence emission spectra of the SYTOX Green nucleic acid stain (S-7020) bound to DNA.

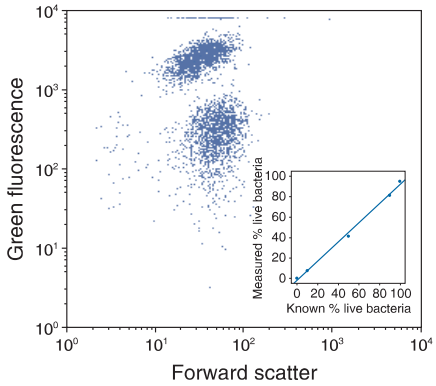

Figure 15.11 Quantitative flow cytometric analysis of *Escherichia coli* viability using the SYTOX Green nucleic acid stain (S-7020). A bacterial suspension containing an equal number of live and isopropyl alcohol–killed *E. coli* was stained with SYTOX Green and analyzed using excitation at 488 nm. A bivariate frequency distribution for forward light scatter versus log fluorescence intensity (collected with a 510 nm longpass optical filter) shows two clearly distinct populations. When live and dead bacteria were mixed in varying proportions, a linear relationship between the population numbers and the actual percentage of live cells in the sample was obtained (see inset).

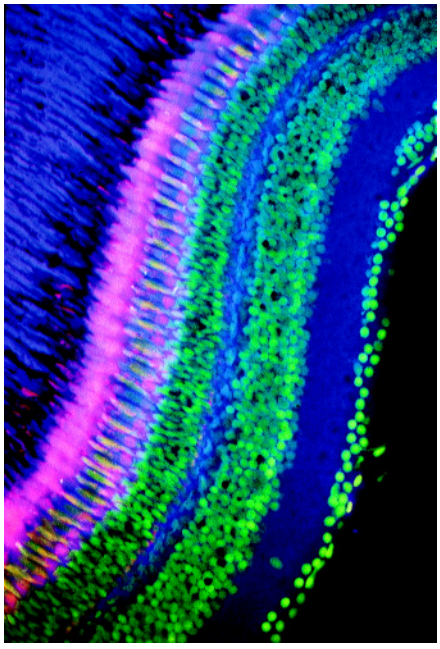

Figure 15.12 A frozen section of zebrafish retina stained with mouse monoclonal antibody FRet 43 in conjunction with Texas Red-X goat anti–mouse IgG (T-6390), Alexa Fluor 350 wheat germ agglutinin (W-11263) and SYTOX Green nucleic acid stain (S-7020).

stain is the high-throughput screening of bacteria for antibiotic susceptibility by fluorescence microscopy, by flow cytometry or in a fluorescence microplate reader.[61] The SYTOX Green stain is also used in our Vybrant Tumor Necrosis Factor (TNF) Assay Kit [62] (V-23100).

The SYTOX Green nucleic acid stain as a tool for viability assessment is not restricted to bacteria; it is also a very effective cell-impermeant counterstain in eukaryotic systems (Section 8.6). It can be used in conjunction with blue- and red-fluorescent labels for multiparametric analyses in fixed cells and tissue sections (Figure 8.7, Figure 15.12, Figure 15.13). Furthermore, it should be possible to combine the SYTOX Green nucleic acid stain with one of the membrane-permeant nucleic acid stains in our SYTO Red-, Blue- or Orange-Fluorescent Nucleic Acid Stain Sampler Kits (S-11340, S-11350, S-11360) for two-color visualization of dead and live cells.

Like the SYTOX Green reagent, our SYTOX Orange and SYTOX Blue nucleic acid stains (S-11368, S-11348) are high-affinity nucleic acid stains that only penetrate cells with compromised plasma membranes. The SYTOX Orange nucleic acid stain (S-11368, Figure 11.19) has absorption/emission maxima of 547/570 nm when bound to DNA and is optimally detected using filters appropriate for rhodamine dyes (Table 24.8). As with the other SYTOX dyes, the SYTOX Orange stain is virtually nonfluorescent except when bound to nucleic acids and can be used to detect cells that have compromised membranes without a wash step.

Our SYTOX Blue nucleic acid stain labels both DNA and RNA with extremely bright fluorescence centered near 480 nm (Figure 8.106), making it an excellent fluorescent indicator of cell viability (Figure 15.14). Unlike many blue-fluorescent dyes, the SYTOX Blue stain is efficiently excited by tungsten–halogen lamps and other sources with relatively poor emission in the UV portion of the spectrum. The brightness of the SYTOX Blue complex with nucleic acids allows sensitive detection of stained cells with fluorometers, fluorescence microplate readers, arc-lamp–equipped flow cytometers and epifluorescence microscopes, including those not equipped with UV light–pass optics.

Quantitation of membrane-compromised bacterial cells carried out with the SYTOX Blue stain yields results identical to those obtained in parallel assays using the SYTOX Green stain. And like the SYTOX Green stain, the SYTOX Blue stain does not interfere with bacterial cell growth. Because their emission spectra overlap somewhat, we have found that it is not ideal to use SYTOX Blue stain and green-fluorescent dyes together in the same application, except when the green-fluorescent dye is excited beyond the absorption of the SYTOX Blue dye (e.g., at >480 nm). However, emission of the SYTOX Blue complex with nucleic acids permits clear discrimination from red- and orange-fluorescent probes, facilitating development of multicolor assays with minimal spectral overlap between signals.

Dimeric and Monomeric Cyanine Dyes

The patented dimeric and monomeric cyanine dyes in the TOTO and TO-PRO series (Table 8.2) are essentially nonfluorescent unless bound to nucleic acids and have extinction coefficients 10–20 times greater than that of DNA-bound propidium iodide. Spectra of the nucleic acid–bound dyes cover the entire visible spectrum and into the infrared region (Figure 8.1). These dyes are typically impermeant to the membranes of live cells [63] but brightly stain dead cells that have compromised membranes. The POPO-1 and BOBO-1 dyes may be useful blue-fluorescent dead-cell stains, and the YOYO-3 and TOTO-3 dyes and the corresponding YO-PRO-3 and TO-PRO-3 dyes have excitation maxima beyond 600 nm when bound to DNA. Our JOJO-1 and JO-PRO-1 dyes exhibit orange fluorescence (~545 nm) upon binding to nucleic acids and can be excited with a 532 nm Nd-YAG laser. The LOLO-1 and LO-PRO-1 nucleic acid stains have longer-wavelength fluorescence (~580 nm) and are maximally excited by the 568 nm spectral line of the krypton-ion laser.

One cell viability assay utilizes YOYO-1 fluorescence before and after treatment with digitonin as a measure of the dead cells and total cells, respectively, in the sample.[64] The YOYO-3 dye was used as a stain for dead cells in an assay designed to correlate cell cycle with metabolism in single cells.[65] In addition to their use as dead-cell stains, both the dimeric and monomeric cyanine dyes are also proving useful for staining viruses. TOTO-1 dye–staining and flow cytometric analysis gave better discrimination of live and

dead lactic acid bacteria in several species than did propidium iodide.[66] Viruses stained with the YOYO-1 and POPO-1 dyes have been employed to identify and quantitate bacteria and cyanobacteria in marine microbial communities.[67] The YO-PRO-1 dye has been used to count viruses in marine and freshwater environments by epifluorescence microscopy [68] and is also selectively permeant to apoptotic cells [69] (Section 15.5). TO-PRO-3 (T-3605, Section 15.4) has been utilized to demonstrate transient permeabilization of bacterial by sub-lethal doses of antibiotics.[70]

Our Nucleic Acid Stains Dimer Sampler Kit (N-7565) provides a total of eight cyanine dyes that span the visible spectrum. This kit should be useful when screening dyes for their utility in viability and cytotoxicity assays.

Ethidium and Propidium Dyes

The red-fluorescent, cell-impermeant ethidium and propidium dyes — ethidium bromide (E-1305, E-3565), ethidium homodimer-1 (EthD-1, E-1169; Figure 8.17) and propidium iodide (P-1304, P-3566; FluoroPure grade, P-21493) — can all be excited by the argon-ion laser and are therefore useful for detecting and sorting dead cells by flow cytometry.[71,72] Moreover, these dyes have large Stokes shifts and may be used in combination with fluorescein-based probes (such as calcein, CellTracker Green CMFDA or BCECF) for two-color applications (Figure 15.15, Figure 15.16). Both propidium iodide and ethidium bromide have been extensively used to detect dead or dying cells,[73–77] although ethidium bromide may be somewhat less reliable because it is not as highly charged. EthD-1 and propidium iodide are superior to ethidium bromide for two-color flow cytometric viability assays in which either BCECF AM or calcein AM is used as the live-cell stain because their spectra do not overlap as much with those of the green-fluorescent esterase probes.[21]

With its high affinity for DNA and low membrane permeability,[78–81] EthD-1 is often the preferred red-fluorescent dead-cell indicator. EthD-1 binds to nucleic acids 1000 times more tightly than does ethidium bromide and undergoes about a 40-fold enhancement of fluorescence upon binding.[80,82] When used as a viability indicator, EthD-1 typically does not require a wash step. Also, the high affinity of EthD-1 permits the use of very low concentrations to stain dead cells, thus avoiding the use of large quantities of the potentially hazardous ethidium bromide or propidium iodide. EthD-1, the dead-cell indicator in our LIVE/DEAD Viability/Cytotoxicity Kit (L-3224, see below), has been used alone [83] or in combination with calcein AM [84] to detect tumor necrosis factor activity

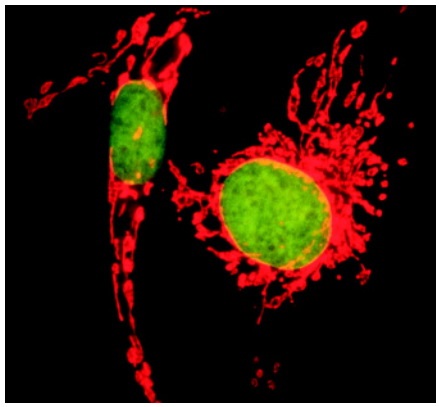

Figure 15.13 The mitochondria of bovine pulmonary artery endothelial cells stained with MitoTracker Red CM-H_2XRos (M-7513). The cells were subsequently fixed, permeabilized and treated with RNase. Then the nuclei were stained with SYTOX Green nucleic acid stain (S-7020). The multiple-exposure photomicrograph was acquired using a fluorescence microscope equipped with bandpass filter sets appropriate for fluorescein and Texas Red dyes.

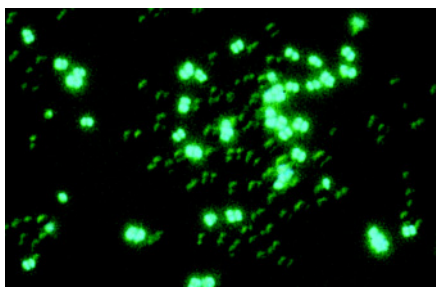

Figure 15.14 A mixed population of live and isopropyl alcohol–killed *Micrococcus luteus* stained with SYTOX Blue nucleic acid stain (S-11348), which does not penetrate intact plasma membranes. Dead cells exhibit bright blue-fluorescent staining. The image was acquired using a longpass optical filter set appropriate for the Cascade Blue dye.

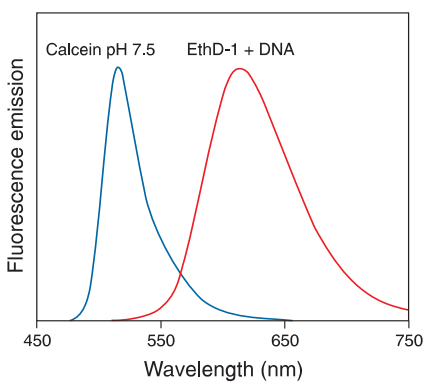

Figure 15.15 Normalized fluorescence emission spectra of calcein (C-481) and DNA-bound ethidium homodimer-1 (EthD-1, E-1169), illustrating the clear spectral separation that allows simultaneous visualization of live and dead eukaryotic cells with Molecular Probes' LIVE/DEAD Viability/Cytotoxicity Kit (L-3224).

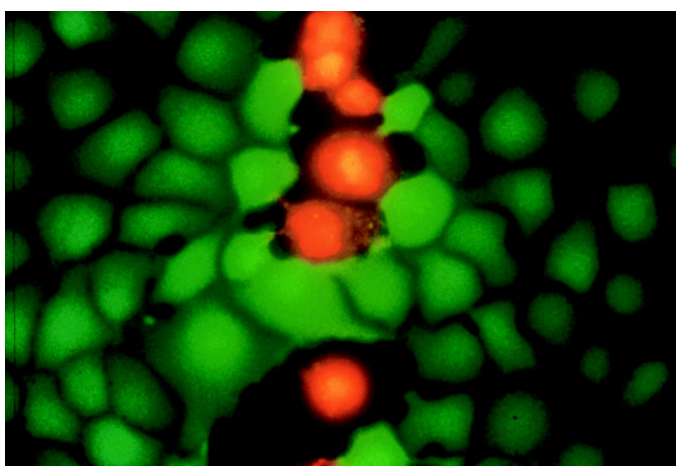

Figure 15.16 Live and dead kangaroo rat (PtK2) cells stained with ethidium homodimer-1 and the esterase substrate calcein AM, both of which are provided in our LIVE/DEAD Viability/Cytotoxicity Kit (L-3224). Live cells fluoresce a bright green, whereas dead cells with compromised membranes fluoresce red-orange.

and to assay neuronal cell death.[74,85,86] Ethidium homodimer-2 (E-3599, Figure 8.18), which under our trademark DEAD Red is used as the necrotic-cell indicator in our LIVE/DEAD Reduced Biohazard Cell Viability Kit #1 (L-7013, Section 15.3), has a particularly low dissociation rate from cellular nucleic acids, permitting its use for selective marking of dead-cell populations that need to be observed over several hours. Our DEAD Red nucleic acid stain has proven useful for determining brain stem lesion size *in vivo* in rats following a neurotoxin injection.[87] Live and dead cells of the yeast-like fungus *Aureobasidium pullulans* have been identified on microscope slides as well as leaf surfaces using CellTracker Blue CMAC (C-2110, Section 14.2) in conjunction with the DEAD Red nucleic acid stain.[88]

Ethidium Monoazide

Ethidium monoazide (E-1374) is a fluorescent photoaffinity label that, after photolysis, binds covalently to nucleic acids in solution and in cells with compromised membranes.[89,90] A mixed population of live and dead cells incubated with this membrane-impermeant dye can be illuminated with a visible-light source, washed, fixed and then analyzed in order to determine the viability of the cells at the time of photolysis.[90] Thus, ethidium monoazide reduces some of the hazards inherent in working with pathogenic samples because, once stained, samples can be treated with fixatives before analysis by fluorescence microscopy or flow cytometry. Immunocytochemical analyses requiring fixation are also compatible with this ethidium monoazide–based viability assay. We have developed an alternative two-color fluorescence-based assay for determining the original viability of fixed samples that employs our cell-permeant, green-fluorescent SYTO 10 and cell-impermeant, red-fluorescent DEAD Red nucleic acid stains; the LIVE/DEAD Reduced Biohazard Cell Viability Kit #1 (L-7013) is described in Section 15.3.

Hexidium Iodide: A Fluorescent Gram Stain

Ethidium bromide is only marginally permeant to cell membranes or bacteria; however, we found that our patented hexidium iodide stain (H-7593) has the right combination of polarity and permeability (Figure 8.16) to allow it to rapidly stain most gram-positive bacteria while being excluded by the less-permeant membranes of most gram-negative bacteria[91] (Figure 15.42). Combining the red-orange–fluorescent hexidium iodide reagent with a green-fluorescent, membrane-permeant nucleic acid stain — as in our LIVE *Bac*Light Bacterial Gram Stain Kit (L-7005, Section 15.3) — enables taxonomic classification of most bacteria in minutes, using a single staining solution, no fixatives and no wash steps. This rapid gram stain assay should be useful in both clinical and research settings. The validity of using hexidium iodide in combination with the SYTO 13 green-fluorescent nucleic acid stain to correctly predict the gram sign of 45 clinically relevant organisms, including several known to be gram variable, has been demonstrated.[91] The method of use of hexidium iodide as a gram stain is described further in Section 15.3.

SYTO Dyes

Our patented SYTO family of dyes, all of which are listed in Table 8.3, are essentially nonfluorescent until they bind to nucleic acids, whereupon their fluorescence quantum yield may increase by 1000-fold or more. These dyes are freely permeant to most cells, although their rate of uptake and ultimate staining pattern may be cell dependent. Their affinity for nucleic acids is moderate and they can be displaced by higher-affinity nucleic acid stains such as SYTOX Green, propidium iodide, the ethidium dimers and all of the monomeric and dimeric nucleic acid stains described above. Because the membrane of intact cells offers a barrier to entry of these higher-affinity nucleic acid stains, it is common to combine, for instance, a green-fluorescent SYTO dye with a red-fluorescent, high-affinity nucleic acid stain such as propidium iodide, one of the ethidium homodimers or TOTO-3 for simultaneous staining of the live- and dead-cell populations. Although the green-fluorescent SYTO dye will still bind to nucleic acids in dead cells, it will be displaced or its fluorescence quenched by the red-fluorescent dye, resulting in a yellow-, orange- or red-fluorescent dead-cell population. This principle is the basis of our LIVE/DEAD *Bac*Light Bacterial Viability Kits (L-7007, L-7012, L-13152; Figure 15.17), our LIVE/DEAD Sperm Viability Kit (L-7011) and our LIVE *Bac*Light Bacterial Gram Stain Kit (L-7005), which are all discussed in Section 15.3. Five sampler kits of the SYTO dyes (S-7554, S-7572, S-11340, S-11350, S-11360) provide a total of 32 SYTO dyes with emission maxima that range from 441 nm to 678 nm. Each of the SYTO dyes is also available individually (Section 8.1). The SYTO 13 green-fluorescent nucleic acid stain (S-7575) has been used in combination with:

- Ethidium bromide for studies of tissue cryopreservation[92]
- Hexidium iodide for simultaneous viability and gram sign of clinically relevant bacteria[91]
- Ethidium homodimer-1 for quantitation of neurotoxicity[74,93]
- Propidium iodide to detect the effects of surfactants on *Escherichia coli* viability[94]

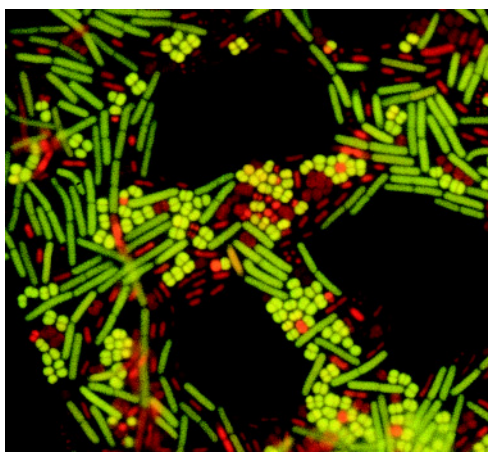

Figure 15.17 A mixed population of live and isopropyl alcohol–killed *Micrococcus luteus* and *Bacillus cereus* stained with the LIVE/DEAD *Bac*Light Bacterial Viability Kit (L-7007, L-7012). Bacteria with intact cell membranes exhibit green fluorescence, whereas bacteria with damaged membranes exhibit red fluorescence. Prior to imaging, the bacteria were placed onto a polycarbonate filter and immersed in *Bac*Light mounting oil. This multiple exposure image was acquired with a triple-bandpass optical filter set appropriate for simultaneous imaging of DAPI, fluorescein and Texas Red dyes.

FUN 1 and FUN 2: Unique Stains for Assessing Viability of Yeast and Fungi

Our patented FUN 1 and FUN 2 dyes (F-7030, F-13150) are structurally related to the SYTO dyes; however, both dyes contain substituents (Figure 12.42) that apparently make them chemically reactive with intracellular components of yeast, provided that the yeast are metabolically active. The FUN 1 and FUN 2 stains are freely taken up by several species of yeast and fungi and converted from a diffusely distributed pool of yellow-green–fluorescent intracellular stain into compact red-orange– or yellow-orange–fluorescent intravacuolar structures, respectively (Figure 15.36). Conversion of the FUN 1 and FUN 2 dyes to products with longer-wavelength emission (Figure 15.35) requires both plasma membrane integrity and metabolic capability. Only metabolically active cells are marked clearly with fluorescent intravacuolar structures, while dead cells exhibit extremely bright, diffuse, yellow-green fluorescence.[60,95] The FUN 1 cell stain is also available as a component in our LIVE/DEAD Yeast Viability Kit (L-7009, Section 15.3).

7-Aminoactinomycin D

7-AAD (7-aminoactinomycin D, A-1310; Section 15.4) is a fluorescent intercalator that undergoes a spectral shift upon association with DNA. 7-AAD/DNA complexes can be excited by the argon-ion laser and emit beyond 610 nm (Figure 8.32), making this nucleic acid stain useful for multicolor fluorescence microscopy, confocal laser-scanning microscopy and flow cytometry. 7-AAD appears to be generally excluded from live cells, although it has been reported to label the nuclear region of live cultured mouse L cells and salivary gland polytene chromosomes of *Chironomus thummi thummi* larvae.[96] 7-AAD is also a useful marker for apoptotic cell populations (Section 15.5) and has been utilized to discriminate dead cells from apoptotic and live cells.[97] In addition, 7-AAD can be used in combination with conjugates of R-phycoerythrin (Section 6.4) in three-color flow cytometry protocols.

Viability/Cytotoxicity Assays That Measure Oxidation or Reduction

Metabolically active cells can oxidize or reduce a variety of probes, providing a measure of cell viability and overall cell health. This measure of viability is distinct from that provided by probes designed to detect esterase activity or cell permeability. Detecting oxidative activity and reactive oxygen species (ROS) in cells is also discussed in Section 19.2.

High-Purity Resazurin

Resazurin (R-12204, Figure 15.18) has been extensively used as an oxidation–reduction indicator to detect bacteria and yeast in broth cultures and milk,[98,99] to assess the activity of sperm [100,101] and to assay bile acids [102,103] and triglycerides.[104] Resazurin reduction also occurs with other mammalian cells, including neurons,[105] corneal endothelial cells,[106] lymphocytes, lymphoid tumor cells and hybridoma cells.[107] Furthermore, resazurin has been used in high-throughput screening assays for compounds that act against *Mycobacterium tuberculosis*.[108] However, correlation of the results obtained with resazurin and bioluminescent assays for ATP has been reported to be poor.[109] Recently, resazurin has been sold under the name alamarBlue (a trademark of AccuMed International, Inc.) and has been reported to be useful for quantitatively measuring cell-mediated cytotoxicity,[110] cell proliferation [111,112] and mitochondrial metabolic activity is isolated neural tissue.[113] Several of our bibliography references on resazurin describe studies in which the alamarBlue reagent was the form of resazurin utilized. Our resazurin has been purified to remove fluorescent and colored products typically found in other commercial resazurin preparations, including the alamarBlue reagent.

Dodecyl Resazurin: A Superior Probe for Cell Metabolic Studies

Dodecyl resazurin (C_{12}-resazurin), which is available only as a component of our Vybrant Cell Metabolic Assay Kit (V-23110, Section 15.3) has several properties that make it superior to resazurin (and alamarBlue) for detecting metabolic activity in cells:

- C_{12}-resazurin is freely permeant to the membranes of most cells.
- Less C_{12}-resazurin is required for equivalent sensitivity.
- Unlike resazurin, which yields a product (resorufin) that rapidly leaks from viable cells, the product of reduction of C_{12}-resazurin — C_{12}-resorufin — is relatively well-retained by single cells, permitting flow cytometric assay of cell metabolism and viability on a single-cell basis (Figure 15.30).
- The fluorescence developed by reduction of C_{12}-resazurin is directly proportional to cell number, and the assay is capable of detecting very low numbers of cells, even in a high-throughput microplate-based assay (Figure 15.31).

Dihydrorhodamines and Dihydrofluoresceins

Fluorescein, rhodamine and various other dyes can be chemically reduced to colorless, nonfluorescent leuco dyes. These "dihydro" derivatives are readily oxidized back to the parent dye by some reactive oxygen species (Section 19.2) and thus can serve as fluorogenic probes for detecting oxidative activity in cells and tissues.[114–116] Because reactive oxygen species are produced by live but not dead cells, fluorescent oxidation products that are retained in cells can be used as viability indicators for single cells or cell suspensions. Some probes that are useful for detecting oxidative activity in metabolically active cells include:

- H_2DCFDA [117] (2′,7′-dichlorodihydrofluorescein diacetate, D-399, Figure 19.6), carboxy-H_2DCFDA (5-(and-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate, C-400) and the acetoxymethyl ester of H_2DCFDA (C-2938, Figure 15.79), all of which require both intracellular deacetylation and oxidation to yield green-fluorescent products [118–120]
- CM-H_2DCFDA (chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester, C-6827), which is analogous to

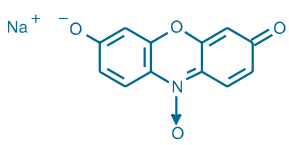

Figure 15.18 R-12204 resazurin, sodium salt.

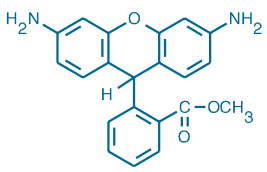

Figure 15.19 D-632 dihydrorhodamine 123.

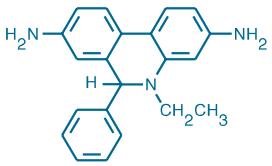

Figure 15.20 D-1168 dihydroethidium.

Assay Kits for Cell Viability and Proliferation

Molecular Probes has developed what are undoubtedly the best and easiest to use kits for measuring cell viability, cytotoxicity and proliferation. These are discussed in Sections 15.3 and 15.4. Our premiere products include:

- LIVE/DEAD Kits for eukaryotic cells (including sperm), bacteria and yeast
- LIVE/DEAD Reduced Biohazard Kits for assessing the original viability of a cell population following fixation
- LIVE/DEAD Cell-Mediated Cytotoxicity Kit for detecting killing of target cells by effector cells
- The CyQUANT Cell Proliferation Assay Kit, an outstanding product for easy cell counting
- Reagents and kits for detecting incorporation of 5-bromo-2'[prime]-deoxyuridine (BrdU) during cell division

H_2DCFDA, except that it forms a mildly thiol-reactive fluorescent product after oxidation by metabolically active cells (Figure 19.9), permitting significantly longer-term measurements [121]
- Dihydrocalcein AM (D-23805), our newest dihydrofluorescein derivative, which is converted intracellularly to calcein, a green-fluorescent dye with superior cell retention (Figure 15.3)
- Dihydrorhodamine 123 (D-632, D-23806; Figure 15.19) and dihydrorhodamine 6G (D-633), which are oxidized in viable cells to the mitochondrial stains rhodamine 123 [122–125] and rhodamine 6G,[126,127] respectively
- Dihydroethidium (also known as hydroethidine; D-1168, D-11347, D-23107; Figure 15.20), which forms the nucleic acid stain ethidium following oxidation [128,129] and has proven useful for detecting the viability of intracellular parasites [130]
- Luminol (L-8455), which is useful for chemiluminescence-based detection of oxidative events in cells rich in peroxidases, including granulocytes [131–134] and spermatozoa [135]

These probes are all described in more detail in Section 19.2, which includes products for assaying oxidative activity in live cells and tissues.

RedoxSensor Red CC-1 Stain

RedoxSensor Red CC-1 stain (R-14060) is a unique probe whose fluorescence localization appears to be based on a cell's cytosolic redox potential. Scientists at Molecular Probes have found that RedoxSensor Red CC-1 stain passively enters live cells. Once inside, the nonfluorescent probe is either oxidized in the cytosol to a red-fluorescent product (excitation/emission maxima ~540/600 nm), which then accumulates in the mitochondria, or the probe is transported to the lysosomes, where it is oxidized. The differential distribution of the oxidized product between mitochondria and lysosomes appears to depend on the redox potential of the cytosol.[136] In proliferating cells, mitochondrial staining predominates; whereas in contact-inhibited cells, the staining is primarily lysosomal. The best method we have found to quantitate the distribution of the oxidized product is to use the mitochondrion-selective MitoTracker Green FM stain (M-7514, Section 12.2) in conjunction with RedoxSensor Red CC-1 stain.

Tetrazolium Salts

Tetrazolium salts are widely used for detecting redox potential of cells for viability, cytotoxicity and proliferation assays.[137–142] Following reduction, these water-soluble, colorless compounds form uncharged, brightly colored but nonfluorescent formazans. Several of the formazans precipitate out of solution and are useful for histochemical localization of the site of reduction or, after solubilization in organic solvent, for quantitation by standard spectrophotometric techniques.

Reduction of MTT (M-6494) remains the most common assay for tetrazolium salt–based viability testing.[143–145] Molecular Probes' convenient Vybrant MTT Cell Proliferation Assay Kit (V-13154, Section 15.4) provides a simple method for determining cell number using standard microplate absorbance readers. MTT has also been used to measure adhesion of HL60 leukemia cells onto endothelial cells.[146] In addition to dehydrogenases, MTT is reduced by glutathione *S*-transferase (GST).[147] Therefore, MTT may not always be a reliable cell viability probe in cells treated with compounds that affect GST activity.

Unlike MTT's purple-colored formazan product, the extremely water-soluble, orange-colored formazan product of XTT (X-6493) does not require solubilization prior to quantitation, thereby reducing the assay time in many viability assay protocols. Moreover, the sensitivity of the XTT reduction assay is reported to be similar to or better than that of the MTT reduction assay.[138] The XTT reduction assay is particularly useful for high-throughput screening of antiviral and antitumor agents and for assessing the effect of cytokines on cell proliferation.[142,148–151] NBT (N-6495) forms a deep blue–colored precipitate that is commonly used to indicate oxidative metabolism.[152,153]

Other Viability/Cytotoxicity Assay Methods

A viable cell contains an ensemble of ion pumps and channels that maintain both intracellular ion concentrations and transmembrane potentials. Active maintenance of ion

gradients ceases when the cell dies, and this loss of activity can be assessed using potentiometric dyes, acidotropic probes, Ca^{2+} indicators [117] (Chapter 20) and pH indicators [117] (Chapter 21).

Potentiometric Dyes

Molecular Probes makes a variety of dyes for detecting transmembrane potential gradients (Chapter 23), including several cationic probes that accumulate in the mitochondria of metabolically active cells (Section 12.2). The mitochondrion-selective rhodamine 123 [117] (R-302; FluoroPure grade, R-22420) has been used to assess the viability of lymphocytes,[154] human fibroblasts,[155] Simian virus–transformed human cells [156] and bacteria; [157] however, rhodamine 123 is not taken up well by gram-negative bacteria.[157] Rhodamine 123 has also been used in combination with propidium iodide (P-1304, P-3566; FluoroPure grade, P-21493) for two-color flow cytometric viability assessment.[158]

The methyl and ethyl esters of tetramethylrhodamine (T-668, T-669) accumulate in the mitochondria of healthy cells in an amount related to the membrane potential. The dyes are nontoxic and highly fluorescent and do not form aggregates or display binding-dependent changes in their fluorescence efficiency, permitting continuous monitoring of cell heath.[159]

Other potential-sensitive dyes that have proven useful in viability studies include several fast-response styryl dyes and slow-response oxonol and carbocyanine dyes. The fast-response styryl dyes such as di-4-ANEPPS (D-1199, Section 23.2) give relatively large fluorescence response to potential changes. Di-4-ANEPPS was used for rapid measurement of toxicity in frog embryos.[160] The symmetrical bis-oxonol dyes [117] (B-413, B-438; Section 23.3) have been used for viability assessment by flow cytometry [161–163] and imaging. These slow-response dyes have also been employed to determine antibiotic susceptibility of bacteria by flow cytometry.[164,165]

The green-fluorescent cyanine dye JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide, T-3168; Figure 23.13) exists as a monomer at low concentrations or at low membrane potential; however, at higher concentrations (aqueous solutions above 0.1 μM) or higher potentials, JC-1 forms red-fluorescent "J-aggregates" (Figure 12.21, Figure 12.22, Figure 23.14) that exhibit a broad excitation spectrum and an emission maximum at ~590 nm (Figure 23.15). JC-1 has been used to investigate apoptosis,[74,166,167] as well as mitochondrial poisoning, uncoupling and anoxia.[168] The ability to make ratiometric emission measurements with JC-1 makes this probe particularly useful for monitoring changes in cell health. We have also discovered another mitochondrial marker, JC-9 (3,3′-dimethyl-β-naphthoxazolium iodide, D-22421; Figure 23.18), with similar potential-dependent spectroscopic properties.

Acidotropic Stains

Membrane-bound proton pumps are used to maintain low pH within the cell's acidic organelles. Our complete selection of stains for lysosomes and other acidic organelles, including LysoTracker and LysoSensor probes, is described in Section 12.3.

The lysosomal stain neutral red (N-3246), which was first used for viability measurements by Ehrlich in 1894, has been employed in numerous cytotoxicity, cell proliferation and adhesion assays.[169–174] Although usually used as a chromophoric probe, neutral red also fluoresces at ~640 nm in viable cells and has been detected using a fluorescence microplate reader.[175] Furthermore, the fluorescence of neutral red and BCECF AM (or SYTOX Green nucleic acid stain) can be measured simultaneously using a single excitation wavelength of 488 nm,[175] suggesting that neutral red may be an effective probe for multicolor flow cytometric determination of cell viability. Our neutral red is highly purified to reduce contaminants that might interfere with these observations.

Acridine orange (A-1301, A-3568) concentrates in acidic organelles in a pH-dependent manner. The metachromatic green or red fluorescence of acridine orange has been used to assess islet viability [176] and bacterial spore viability [177] and to monitor physiological activity in *Escherichia coli*.[178]

LysoTracker Green DND-26 (L-7526, Section 12.3) was used in a fluorometric assay of cryopreserved sperm to demonstrate both acrosomal integrity and sperm viability.[179] Of several methods used, LysoTracker Green DND-26 staining was the quickest and easiest to use and gave excellent correlation with the SYBR 14 staining method used in our LIVE/DEAD Sperm Viability Kit (L-7011, see below). When observed with a fluorescence microscope, sperm appeared to lose their green-fluorescent LysoTracker Green DND-26 staining and instead exhibit red-fluorescent propidium iodide staining within about 30 seconds after motility ceased.[180,181]

Fluorescent Glucose Analogs

Measurements of glucose uptake can be used to assess viability in a variety of organisms. 2-NBD-deoxyglucose (2-NBDG, N-13195) has been used to monitor glucose uptake in living pancreatic β-cells,[182] the yeast *Candida albicans* [183] and the bacteria *Escherichia coli*.[184–186] Molecular Probes also offers the fluorescent nonhydrolyzable glucose analog 6-NBD-deoxyglucose (6-NBDG, N-23106). Using this probe, researchers have studied glucose uptake and transport in isolated cells [187–189] and intact tissues.[190] Although sensitive to its environment NBD fluorescence typically displays excitation/emission maxima of ~465/540 nm and can be visualized using optical filters designed for fluorescein.

Fluorescent Antibiotics and Related Probes

Fluorescent Polymyxins

Polymyxin B is a cyclic cationic peptide antibiotic that binds to the lipopolysaccharide (LPS) component of the outer membrane of gram-negative bacteria and increases its permeability to lysozyme and hydrophobic compounds. Fluorescent BODIPY FL (P-13235), Oregon Green 514 (P-13236), Texas Red-X (P-13237) and dansyl (P-13238) derivatives of polymyxin B are available from Molecular Probes. Dansyl polymyxin B, which fluoresces weakly when free in solution, becomes highly fluorescent (excitation/emission ~340/485 nm) upon binding to intact cells or LPS.[191] The binding of dansyl polymyxin B to LPS can be displaced by a variety of polycationic antibiotics, as well as by Mg^{2+}. Consequently, dansyl polymyxin B displacement experiments can be used to assess the binding of various compounds, such as antibiotics and macrophage cationic proteins, to LPS and intact bacterial cells.[192–194] Dansyl polymyxin has also been used to localize regions of high anionic lipid content in both sperm [195] and aggregated human platelets,[196] and to analyze the morphology of lipid bilayers in preparations of acetylcholine receptor clusters from rat myotubules.[197] Green-fluorescent BODIPY FL

and Oregon Green 514 polymyxin B and red-fluorescent Texas Red-X polymyxin B provide additional color options for these experiments. Our fluorescent LPS are described in Section 13.3 and Section 16.1.

Fluorescent Penicillin Analogs

Penicillin-binding proteins, which are present on the cytoplasmic membranes of eubacteria, can be detected with our BOCILLIN FL penicillin (B-13233) and BOCILLIN 650/665 penicillin (B-13234). BOCILLIN FL penicillin, synthesized from penicillin V and BODIPY FL dye (which is spectrally similar to fluorescein), has been used to determine the penicillin-binding protein profiles of *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*, which are similar to binding profiles reported by researchers using radioactively labeled penicillin V.[198] Fluorescently labeled penicillin has also been used for direct labeling and rapid detection of whole *E. coli* and *Bacillus licheniformis* [199] and of *Enterobacter pneumoniae*.[200] Fluorescent penicillin analogs can also be used to detect penicillin-binding proteins separated by gel electrophoresis with sensitivity in the low nanogram range.[201]

BODIPY FL Vancomycin

BODIPY FL vancomycin (V-23108), which contains a single BODIPY FL dye per vancomycin molecule, is a green-fluorescent analog of this important antibiotic, which is active against gram-positive bacteria, including enterrococci. Although its biological activity has not yet been reported, BODIPY FL vancomycin may be useful for detecting vancomycin binding sites and transport, for fluorescence polarization (FP, see Section 1.4) assays of vancomycin concentration in serum [202–204] and for the study and detection of vancomycin-resistant enterococci [205] (VRE, Section 15.6).

Antimalarial Agents

The phosphonate antibiotics FR-31564 (fosmidomycin, F-23103) and FR-900098 (F-23104) are effective antimalarial agents that function by blocking a mevalonate-independent pathway of isoprene synthesis.[206,207] The antibiotic activity of fosmidomycin is potentiated by glucose 1-phosphate.[208,209] Both products are also active against several gram-negative bacteria.[210,211]

References

1. 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Data Table — 15.2 Viability and Cytotoxicity Assay Reagents

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
A-1301	301.82	L	H_2O, EtOH	500	53,000	526	H_2O/DNA	1, 2
A-3568	301.82	RR,L	H_2O	500	53,000	526	H_2O/DNA	1, 2, 3
B-1150	~615	F,D	DMSO	<300		none		4, 5
B-1170	~615	F,D	DMSO	<300		none		4, 5
B-3051	~615	F,D	DMSO	<300		none		3, 4, 5
B-13233	661.46	F,D,L	H_2O, DMSO	504	68,000	511	MeOH	
B-13234	653.44	F,D,L	DMSO	646	78,000	659	MeOH	
C-195	460.40	F,D	DMSO	<300		none		6
C-369	529.29	F,D	DMSO	<300		none		7
C-400	531.30	F,D	DMSO, EtOH	290	5,600	none	MeCN	8
C-1354	532.46	F,D	DMSO	<300		none		9
C-1361	460.40	F,D	DMSO	<300		none		6
C-1362	460.40	F,D	DMSO	<300		none		6
C-1429	465.41	F,D,L	DMSO	322	14,000	435	MeOH	10
C-1430	994.87	F,D	DMSO	<300		none		11
C-2925	464.86	F,D	DMSO	<300		none		6
C-2938	675.43	F,D,AA	DMSO	291	5,700	none	MeOH	8
C-3099	994.87	F,D	DMSO	<300		none		3, 11
C-3100	994.87	F,D	DMSO	<300		none		11
C-6826	499.95	F,D	DMSO	<350		none		12
C-6827	577.80	F,D,AA	DMSO	287	9,100	none	MeOH	8
C-7025	464.86	F,D	DMSO	<300		none		6
C-13196	560.52	F,D	DMSO	<300		none		13
D-399	487.29	F,D	DMSO, EtOH	258	11,000	none	MeOH	8
D-632	346.38	F,D,L,AA	DMF, DMSO	289	7,100	none	MeOH	14, 15
D-633	444.57	F,D,L,AA	DMF, DMSO	296	11,000	none	MeOH	14, 15
D-1168	315.42	FF,L,AA	DMF, DMSO	355	14,000	see Notes	MeCN	14, 16
D-11347	315.42	FF,L,AA	DMF, DMSO	355	14,000	see Notes	MeCN	14, 16
D-22421	532.38	D,L	DMSO, DMF	522	143,000	535	$CHCl_3$	17
D-23107	315.42	FF,D,L,AA	DMSO	355	14,000	see Notes	MeCN	16, 18
D-23805	1068.95	F,D	DMSO	285	5,800	none	MeCN	19
D-23806	346.38	F,D,L,AA	DMSO	289	7,100	none	MeOH	15, 18
E-1169	856.77	F,D,L	DMSO	528	7,000	617	H_2O/DNA	1, 20, 21
E-1305	394.31	L	H_2O, DMSO	518	5,200	605	H_2O/DNA	1, 22
E-1374	420.31	F,LL	DMF, EtOH	462	5,400	625	pH 7	23
E-3565	394.31	RR,L	H_2O	518	5,200	605	H_2O/DNA	1, 3, 22
E-3599	1292.71	F,D,L	DMSO	535	8,000	624	H_2O/DNA	1, 3, 20, 21
F-1303	416.39	F,D	DMSO	<300		none		6
F-7030	528.84	F,D,L	DMSO	508	71,000	none	pH 7	3, 24
F-13150	387.33	F,D,L	DMSO	465	81,000	none	pH 7	3, 25
F-23103	205.08	F,D,L	H_2O	<300		none		
F-23104	219.11	F,D,L	H_2O	<300		none		
H-7593	497.42	L	DMSO	518	3,900	600	H_2O/DNA	1, 26
L-8455	177.16	D,L	DMF	355	7,500	411	MeOH	27
M-6494	414.32	D,L	H_2O, DMSO	375	8,300	none	MeOH	28, 29
N-3246	288.78	D,L	H_2O, EtOH	541	39,000	640	see Notes	30
N-6495	817.65	D,L	H_2O, DMSO	256	64,000	none	MeOH	28
N-13195	342.26	F,L	H_2O	466	20,000	540	MeOH	31
N-23106	342.26	F,L	H_2O	467	22,000	540	MeOH	31
O-6151	496.38	F,D	DMSO	<300		none		32
P-1304	668.40	L	H_2O, DMSO	535	5,400	617	H_2O/DNA	1, 33
P-3566	668.40	RR,L	H_2O	535	5,400	617	H_2O/DNA	1, 3, 33

Data Table — 15.2 Viability and Cytotoxicity Assay Reagents — continued

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
P-12880	625.46	F,D	DMSO	<300		none		34
P-21493	668.40	L	H_2O, DMSO	535	5,400	617	H_2O/DNA	35
R-302	380.83	F,D,L	MeOH, DMF	507	101,000	529	MeOH	
R-12204	251.17	L	H_2O, MeOH	604	60,000	none	MeOH	36
R-14060	434.41	F,D,L,AA	DMSO	239	52,000	none	MeOH	14, 37
R-22420	380.83	F,D,L	MeOH, DMF	507	101,000	529	MeOH	35
S-1129	518.43	F,D	DMSO	<300		none		38
S-7020	~600	F,D,L	DMSO	504	67,000	523	H_2O/DNA	1, 3, 39, 40
S-7575	~400	F,D,L	DMSO	488	74,000	509	H_2O/DNA	1, 3, 39, 40
S-11348	~400	F,D,L	DMSO	445	38,000	470	H_2O/DNA	1, 3, 39, 40
S-11368	~500	F,D,L	DMSO	547	79,000	570	H_2O/DNA	1, 3, 39, 40
T-668	500.93	F,D,L	DMSO, MeOH	549	115,000	573	MeOH	
T-669	514.96	F,D,L	DMSO, EtOH	549	109,000	574	MeOH	
T-3168	652.23	D,L	DMSO, DMF	514	195,000	529	MeOH	41
V-23108	1723.35	F,D,L	H_2O, DMSO	504	68,000	511	MeOH	
X-6493	674.53	D	H_2O, DMSO	286	15,000	none	MeOH	42

For definitions of the contents of this data table, see "How to Use This Book" on page viii.

Notes

1. Spectra represent aqueous solutions of nucleic acid-bound dye. EC values are derived by comparing the absorbance of the nucleic acid–bound dye with that of free dye in a reference solvent (H_2O or MeOH).
2. Acridine orange bound to RNA has Abs ~460 nm, Em ~650 nm (Methods Cell Biol 41, 401 (1994); Cytometry 2, 201 (1982)).
3. This product is supplied as a ready-made solution in the solvent indicated under **Soluble**.
4. MW value is approximate. BCECF, AM is a mixture of molecular species. Lot-specific average MW values are printed on product labels.
5. BCECF AM is colorless and nonfluorescent until converted to BCECF (B-1151, Section 21.2) by acetoxymethyl ester hydrolysis.
6. This compound is converted to a fluorescent product with pH-dependent spectra similar to C-1904 (Section 21.2) after acetate hydrolysis.
7. C-369 is converted to a fluorescent product (C-368, Section 21.3) after acetate hydrolysis.
8. Dihydrofluorescein diacetates are colorless and nonfluorescent until both the acetates are hydrolyzed and the products are subsequently oxidized to fluorescein derivatives. The materials contain less than 0.1% of oxidized derivative when initially prepared. The end products from C-400, C-2938, C-6827 and D-399 are 2′,7′-dichlorofluorescein derivatives with spectra similar to C-368 (Section 21.3).
9. Hydrolysis of the acetate and acetoxymethyl ester groups of C-1354 yields C-1359 (Section 1.5).
10. Spectra of C-1429 after acetate hydrolysis are similar to 7-hydroxy-4-methylcoumarin (H-189, Section 10.1).
11. Calcein AM is converted to fluorescent calcein (C-481, Section 14.3) after acetoxymethyl ester hydrolysis.
12. C-6826 is converted to a fluorescent product with spectra similar to C-1270 (Section 21.2) after acetate hydrolysis.
13. C-13196 is converted to a fluorescent product (C-652, Section 21.2) after acetate hydrolysis.
14. This compound is susceptible to oxidation, especially in solution. Store solutions under argon or nitrogen. Oxidation appears to be catalyzed by illumination.
15. D-632, D-23806 and D-633 are essentially colorless and nonfluorescent until oxidized. Oxidation products are R-302 (from D-632 and D-23806) and R-634 (Section 12.2, from D-633).
16. Dihydroethidium has blue fluorescence (Em ~420 nm) until oxidized to ethidium E-1305. The reduced dye does not bind to nucleic acids (FEBS Lett 26, 169 (1972)).
17. JC-9 exhibits long-wavelength J-aggregate emission at ~635 nm in aqueous solutions and polarized mitochondria.
18. This product is supplied as a ready-made solution in DMSO with sodium borohydride added to inhibit oxidation.
19. D-23805 is colorless and nonfluorescent until the AM ester groups are hydrolyzed and the resulting leuco dye is subsequently oxidized. The final product is calcein (C-481, Section 14.3).
20. Although this compound is soluble in water, preparation of stock solutions in water is not recommended because of possible adsorption onto glass or plastic.
21. E-1169 in H_2O: Abs = 493 nm (EC = 9100 $cm^{-1}M^{-1}$). E-3599 in H_2O: Abs = 498 nm (EC = 10,800 $cm^{-1}M^{-1}$). Both compounds are very weakly fluorescent in H_2O. QY increases >40-fold on binding to dsDNA.
22. Ethidium bromide in H_2O: Abs = 480 nm (EC = 5600 $cm^{-1}M^{-1}$), Em = 620 nm (weakly fluorescent). Fluorescence is enhanced >10-fold on binding to dsDNA.
23. E-1374 spectral data are for the free dye. Fluorescence is weak, but intensity increases ~15-fold on binding to DNA. After photocrosslinking to DNA, Abs = 504 nm (EC ~4000 $cm^{-1}M^{-1}$), Em = 600 nm (Nucleic Acids Res 5, 4891 (1978); Biochemistry 19, 3221 (1980)).
24. F-7030 is fluorescent when bound to DNA (Em = 538 nm). Uptake and processing of the dye by live yeast results in red-shifted fluorescence (Em ~590 nm).
25. F-13150 is fluorescent when bound to DNA (Em = 510 nm). Uptake and processing of the dye by live yeast results in red-shifted fluorescence (Em ~550 nm).
26. H-7593 in H_2O: Abs = 482 nm (EC = 5500 $cm^{-1}M^{-1}$), Em = 625 nm (weakly fluorescent).
27. This compound emits chemiluminescence upon oxidation in basic aqueous solutions. Luminescence emission peak is at 425 nm.
28. Enzymatic reduction products are water-insoluble formazans with Abs = 505 nm (M-6494) and 605 nm (N-6495) after solubilization in DMSO or DMF. See literature sources for further information (Histochemistry 76, 381 (1982); Prog Histochem Cytochem 9, 1 (1976)).
29. M-6494 also has Abs = 242 nm (EC = 21,000 $cm^{-1}M^{-1}$) in MeOH.
30. Spectra of N-3246 are pH-dependent (pK_a ~6.7). Data reported are for 1:1 (v/v) EtOH/1% acetic acid.
31. NBD derivatives are almost nonfluorescent in water. QY and τ increase and Em decreases in aprotic solvents and other nonpolar environments relative to water (Biochemistry 16, 5150 (1977); Photochem Photobiol 54, 361 (1991)).
32. O-6151 is converted to a fluorescent product (Abs = 492 nm (EC = 88,000 $cm^{-1}M^{-1}$), Em = 517 nm in H_2O, pH 9.0) after acetate hydrolysis.
33. Propidium iodide in H_2O: Abs = 493 nm (EC = 5900 $cm^{-1}M^{-1}$), Em = 636 nm (weakly fluorescent). Fluorescence is enhanced >10-fold on binding to dsDNA.
34. P-12880 is converted to a fluorescent product (P-12925, Section 15.6) after acetate hydrolysis.
35. This product is specified to equal or exceed 98% analytical purity by HPLC.
36. Enzymatic reduction of resazurin yields resorufin (R-363, Section 10.1).
37. R-14060 is colorless and nonfluorescent until oxidized. The spectral characteristics of the oxidation product (2,3,4,5,6-pentafluorotetramethylrosamine) are similar to those of T-639 (Section 12.2).
38. S-1129 is converted to a fluorescent product (F-1130, Section 21.2) after acetate hydrolysis.
39. This product is essentially nonfluorescent except when bound to DNA or RNA.
40. MW: The preceding ~ symbol indicates an approximate value, not including counterions.
41. JC-1 forms J-aggregates with Abs/Em = 585/590 nm at concentrations above 0.1 μM in aqueous solutions (pH 8.0) (Biochemistry 30, 4480 (1991)).
42. Enzymatic reduction product is a water-soluble formazan, Abs = 475 nm.

Cat #	Product Name	Unit Size
A-1301	acridine orange	1 g
A-3568	acridine orange *10 mg/mL solution in water*	10 mL
B-1150	2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF, AM)	1 mg
B-1170	2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF, AM) *special packaging*	20 x 50 µg
B-3051	2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF, AM) *1 mg/mL solution in dry DMSO*	1 mL
B-13233	BOCILLIN™ FL penicillin, sodium salt	1 mg
B-13234	BOCILLIN™ 650/665 penicillin, sodium salt	1 mg
C-1430	calcein, AM	1 mg
C-3099	calcein, AM *1 mg/mL solution in dry DMSO*	1 mL
C-3100	calcein, AM *special packaging*	20 x 50 µg
C-1429	calcein blue, AM	1 mg
C-400	5-(and-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (carboxy-H_2DCFDA) *mixed isomers*	25 mg
C-2938	6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate, di(acetoxymethyl ester)	5 mg
C-369	5-(and-6)-carboxy-2′,7′-dichlorofluorescein diacetate (carboxy-DCFDA) *mixed isomers*	100 mg
C-1361	5-carboxyfluorescein diacetate (5-CFDA) *single isomer*	100 mg
C-1362	6-carboxyfluorescein diacetate (6-CFDA) *single isomer*	100 mg
C-195	5-(and-6)-carboxyfluorescein diacetate (5(6)-CFDA) *mixed isomers*	100 mg
C-1354	5-carboxyfluorescein diacetate, acetoxymethyl ester (5-CFDA, AM)	5 mg
C-13196	5-(and-6)-carboxynaphthofluorescein diacetate	10 mg
C-2925	CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate)	1 mg
C-7025	CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate) *special packaging*	20 x 50 µg
C-6827	5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA) *mixed isomers* *special packaging*	20 x 50 µg
C-6826	5-(and-6)-chloromethyl SNARF®-1, acetate *mixed isomers* *special packaging*	20 x 50 µg
D-399	2′,7′-dichlorodihydrofluorescein diacetate (2′,7′-dichlorofluorescin diacetate; H_2DCFDA)	100 mg
D-23805	dihydrocalcein, AM *special packaging*	20 x 50 µg
D-1168	dihydroethidium (hydroethidine)	25 mg
D-11347	dihydroethidium (hydroethidine) *special packaging*	10 x 1 mg
D-23107	dihydroethidium (hydroethidine) *5 mM stabilized solution in DMSO*	1 mL
D-632	dihydrorhodamine 123	10 mg
D-23806	dihydrorhodamine 123 *5 mM stabilized solution in DMSO*	1 mL
D-633	dihydrorhodamine 6G	25 mg
D-22421	3,3′-dimethyl-α-naphthoxacarbocyanine iodide (JC-9; $DiNOC_1(3)$)	5 mg
E-1305	ethidium bromide	1 g
E-3565	ethidium bromide *10 mg/mL solution in water*	10 mL
E-1169	ethidium homodimer-1 (EthD-1)	1 mg
E-3599	ethidium homodimer-2 (EthD-2) *1 mM solution in DMSO*	200 µL
E-1374	ethidium monoazide bromide (EMA)	5 mg
F-1303	fluorescein diacetate (FDA)	1 g
F-23103	fosmidomycin, sodium salt (FR-31564)	25 mg
F-23104	FR-900098	25 mg
F-7030	FUN® 1 cell stain *10 mM solution in DMSO*	100 µL
F-13150	FUN® 2 cell stain *10 mM solution in DMSO*	100 µL
H-7593	hexidium iodide	5 mg
L-8455	luminol (3-aminophthalhydrazide)	25 g
M-6494	MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)	1 g
N-3246	neutral red *high purity*	25 mg
N-13195	2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG)	5 mg
N-23106	6-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-6-deoxyglucose (6-NBDG)	5 mg
N-6495	nitro blue tetrazolium chloride (NBT)	1 g
N-7565	Nucleic Acid Stains Dimer Sampler Kit	1 kit
O-6151	Oregon Green® 488 carboxylic acid diacetate (carboxy-DFFDA) *6-isomer*	5 mg
P-12880	5-(pentafluorobenzoylamino)fluorescein diacetate (PFB-FDA)	5 mg
P-13235	polymyxin B, BODIPY® FL conjugate, trifluoroacetic acid salt *mixed species*	100 µg
P-13238	polymyxin B, dansyl conjugate, trifluoroacetic acid salt *mixed species*	100 µg
P-13236	polymyxin B, Oregon Green® 514 conjugate, trifluoroacetic acid salt *mixed species*	100 µg
P-13237	polymyxin B, Texas Red®-X conjugate, trifluoroacetic acid salt *mixed species*	100 µg
P-1304	propidium iodide	100 mg
P-21493	propidium iodide *FluoroPure™ grade*	100 mg
P-3566	propidium iodide *1.0 mg/mL solution in water*	10 mL
R-14060	RedoxSensor™ Red CC-1 *special packaging*	10 x 50 µg
R-12204	resazurin, sodium salt	10 mg
R-302	rhodamine 123	25 mg
R-22420	rhodamine 123 *FluoroPure™ grade*	25 mg

Cat #	Product Name	Unit Size
S-1129	5-sulfofluorescein diacetate, sodium salt (SFDA)	25 mg
S-11350	SYTO® Blue Fluorescent Nucleic Acid Stain Sampler Kit *SYTO® dyes 40-45* *50 µL each*	1 kit
S-7575	SYTO® 13 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-7572	SYTO® Green Fluorescent Nucleic Acid Stain Sampler Kit #1 *SYTO® dyes 11-16* *50 µL each*	1 kit
S-7554	SYTO® Green Fluorescent Nucleic Acid Stain Sampler Kit #2 *SYTO® dyes 20-25* *50 µL each*	1 kit
S-11360	SYTO® Orange Fluorescent Nucleic Acid Stain Sampler Kit *SYTO® dyes 80-85* *50 µL each*	1 kit
S-11340	SYTO® Red Fluorescent Nucleic Acid Stain Sampler Kit *SYTO® dyes 17 and 59-64* *50 µL each*	1 kit
S-11348	SYTOX® Blue nucleic acid stain *5 mM solution in DMSO*	250 µL
S-7020	SYTOX® Green nucleic acid stain *5 mM solution in DMSO*	250 µL
S-11368	SYTOX® Orange nucleic acid stain *5 mM solution in DMSO*	250 µL
T-3168	5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1; $CBIC_2(3)$)	5 mg
T-669	tetramethylrhodamine, ethyl ester, perchlorate (TMRE)	25 mg
T-668	tetramethylrhodamine, methyl ester, perchlorate (TMRM)	25 mg
V-23108	vancomycin, BODIPY® FL conjugate (BODIPY® FL vancomycin)	1 mg
X-6493	XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide)	100 mg

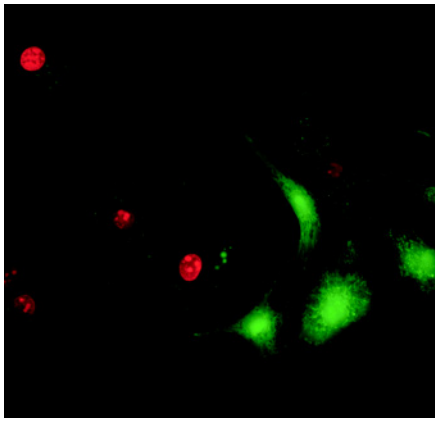

Figure 15.21 A mixture of live and ethanol-killed bovine pulmonary artery epithelial cells stained with the reagents in our LIVE/DEAD Cell Viability/Cytotoxicity Assay Kit (L-3224). Live cells fluoresce bright green, whereas dead cells with compromised membranes fluoresce red-orange.

The LIVE/DEAD Cell Viability/Cytotoxicity Assay Kit (L-3224) is particularly easy to use, gives quantitative results and has a high rate of throughput. No wash steps are required, and definitive results can be obtained using flow cytometry, imaging or a fluorescence-based microplate reader.

15.3 Viability and Cytotoxicity Assay Kits for Diverse Cell Types

This section contains a thorough description of each of our viability and cytotoxicity kits. Many of these assay kits have been developed and patented by Molecular Probes' scientists and are exclusively available from Molecular Probes and its distributors. Fluorometric assays of cell viability and cytotoxicity are easy to perform with the use of a fluorescence microscope, fluorometer, fluorescence microplate reader or flow cytometer,[1] and they offer many advantages over traditional colorimetric and radioactivity-based assays. Also discussed in this section are our unique single-step kits for assessing gram sign and for simultaneously determining gram sign and viability of bacteria.

Viability Assay Kits for Animal Cells

To facilitate use of our unique cell viability and cytotoxicity assay technology, Molecular Probes has developed several important products (Table 15.2) that combine fluorescent reagents to yield, in most cases, two-color discrimination of the population of live cells from the dead-cell population by simply adding the reagents, incubating for a brief period and observing the results without any wash steps required. These facile assays are ideal for high-throughput screening applications and, in most cases, for imaging, fluorometry and flow cytometry. Our unique LIVE/DEAD Reduced Biohazard Kits permit discrimination of the live and dead populations with the analysis performed subsequent to fixation to kill possible pathogens in the sample. Some of our specialized assay kits enable sensitive assays for cell-mediated cytotoxicity, tumor necrosis factor and general cell cytotoxicity through analysis of glucose 6-phosphate dehydrogenase or ATP leakage from cells in bodily fluids. In addition, use of our single-step LIVE *Bac*Light Bacterial Gram Stain Kit or ViaGram Red$^+$ Bacterial Gram Stain and Viability Kit (L-7005, V-7023, see below) overcomes many of the problems inherent in the otherwise labor-intensive, fixation-dependent, gram-stain procedure.

LIVE/DEAD Viability/Cytotoxicity Kit for Animal Cells

Molecular Probes' patented LIVE/DEAD Viability/Cytotoxicity Kit (L-3224) for animal cells provides an exceptionally easy fluorescence-based method for determining viability of adherent or nonadherent cells and for assaying cytotoxicity.[2] The kit comprises two probes: calcein AM and ethidium homodimer-1. Calcein AM (Figure 15.2) is a fluorogenic esterase substrate that is hydrolyzed to a green-fluorescent product (calcein, Figure 14.32); thus, green fluorescence is an indicator of cells that have esterase activity