

15.1 Overview of Probes for Cell Viability, Cell Proliferation and Live-Cell Function

Cell viability, cell proliferation and many important live-cell functions — including apoptosis, cell adhesion, chemotaxis, multidrug resistance, endocytosis, secretion and signal transduction — can be stimulated or monitored with various chemical and biological reagents. Many of these processes lead to changes in intracellular radicals (Chapter 19), free-ion concentrations (Chapter 20, Chapter 21, Chapter 22) or membrane potential (Chapter 23) that can be followed with appropriately responsive fluorescent indicators. This chapter discusses important reagents and assays for detecting these diverse cell processes in live cells. It also includes our important antibodies to cell-cycle-related markers (for example, cyclins and bromodeoxyuridine (BrdU)), which are useful in fixed-cell preparations (Section 15.4). Many of the assays in this chapter can be analyzed on a cell-by-cell basis and some are equally suitable for detection by microscopy, by flow cytometry or with a microplate reader. Almost all of the assays have the capacity for high-throughput analysis.

Our viability and cytotoxicity assay reagents (Section 15.2) and kits (Section 15.3) are principally used to enumerate the proportion of live and dead cells in a population. In contrast, proliferation assays (Section 15.4) are primarily designed to monitor the growth rate of a cell population or to detect daughter cells in a growing population. Fluorescence-based cell viability and proliferation assays are generally less hazardous and less expensive than radioisotopic techniques, more sensitive than colorimetric methods and more convenient than animal testing methods.¹ Unlike ⁵¹Cr-release assays, fluorescence-based assays of cell-mediated cytotoxicity do

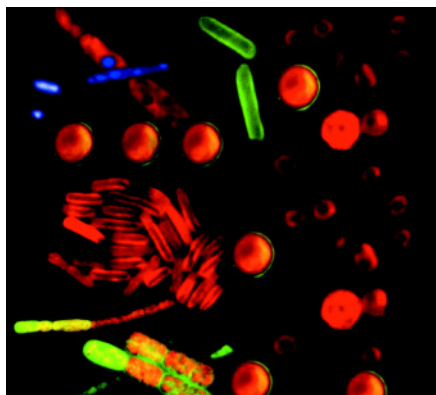


Figure 15.1 Collage of images of cyanobacteria stained with various blue- or green-fluorescent probes to complement the natural red autofluorescence from chlorophyll and phycobilisomes. The round cells are *Synechocystis* sp. (strain PCC 6803), and their membranes were labeled with green-fluorescent BODIPY FL propionic acid (D-2183). The cylindrical cells are *Synechococcus* sp. (strain PCC 7942), stained with blue-fluorescent DAPI (D-1306, D-3571, D-21490). The filamentous cyanobacteria, *Anabaena cylindrica*, were labeled with either the green-fluorescent cytosolic stain, CellTracker Green BODIPY (C-2102), or with the lipophilic membrane stain BODIPY FL (D-2183). Image contributed by Mary Sarcina, Photosynthesis Research Group, Department of Biology, University College, London.

not require large samples, which can be difficult to obtain from patients.² Furthermore, fluorescence-based protocols are more convenient than the trypan blue–exclusion assay. This common colorimetric method for determining cell viability must be completed within 3–5 minutes because the number of blue-staining cells increases with time after addition of the dye.³

Fluorescent dye-based assays for cell viability and cytotoxicity are reliable and easy to perform. Our standalone reagents for these assays are described in Section 15.2, whereas our kits for viability and cytotoxicity analysis are discussed in Section 15.3. Molecular Probes' LIVE/DEAD Viability Assay Kits (Section 15.3) give researchers a choice of viability/cytotoxicity assays suitable for bacteria, fungi or higher eukaryotic cells (Table 15.2). Our LIVE/DEAD Reduced Biohazard Kits (Section 15.3) permit the original viability status of a mixed-cell population to be determined following treatment by aldehyde-based fixatives to kill pathogens. In each case, these viability assay kits provide the reagents and a simple protocol for simultaneous two-color assessment of numbers of live and dead cells. We also offer several proliferation assay kits that enable researchers to rapidly monitor numbers of adherent or nonadherent cells based on the presence of newly replicated DNA, the total nucleic acid content or the total protein content (Section 15.4).

The diversity of live cells and their environments (Figure 15.1) makes it impossible to devise a single viability or enumeration assay applicable to all cell types. Because viability is not easily defined in terms of a single physiological or morphological parameter, it is often desirable to combine several different measures, such as enzymatic activity, membrane permeability and oxidation–reduction (redox) potential. Each assay method has inherent advantages and limitations and may introduce specific biases into the experiment; thus, different applications often call for different approaches.

Apoptosis research is a burgeoning field in which fluorescent probes are having a major impact. Consequently, Section 15.5 focuses on our probes for monitoring apoptosis, including our proprietary reagents for selectively detecting apoptotic cells based on their cell-permeability properties, as well as some exclusive conjugates of annexin V phosphatidylserine-binding protein.

In addition to our probes for cell viability, cell proliferation and apoptosis, several of the reagents for live-cell function described in Section 15.6 can be used to develop assays that measure a particular biochemical parameter of interest. There is a significant overlap between probes for cell viability and probes for live-cell functions. For example, fluorogenic esterase substrates are commonly used to detect viability and proliferation, as well as to monitor cell adhesion, apoptosis and multidrug resistance. Likewise, cell-permeant and cell-impermeant nucleic acid stains are widely applicable to many live-cell function assays. We have organized discussions in this chapter according to several commonly studied cell processes in order to highlight the many published applications for these probes and foster the development of new applications.

References

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2. Hum Immunol 37, 264 (1993);
3. J Histochem Cytochem 33, 77 (1985).