Cell-based Assays 2016

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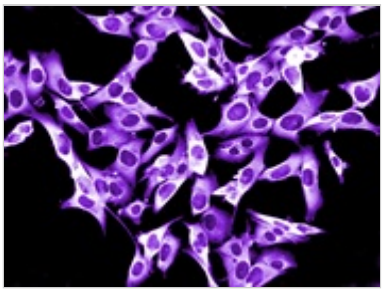

Josh P. Roberts

Counting is important to almost any cell-based manipulation—if only to know how many cells to seed into a well or to normalize an assay. Yet often counting is not enough: Researchers want to know more about those cells—individually or collectively—as a snapshot in time, and often how they faring over time, as well. Viability assays, in their various guises, are often used as first-line barometers for cell health (and inversely, for stress and cytotoxicity). Proliferation assays speak to a culture's inclination to undergo cell division—T cells' response to antigenic stimulation, for example, or inhibition of tumor growth upon introduction of a small molecule.

Although cell count, viability and proliferation are closely related, they—and the assays to measure them—are not necessarily interchangeable.

Here we examine some methods that are used to monitor and characterize cells.

It is alive

To determine cell viability, "what you want to know is how many live cells you have, and how many are dead. Many but not all cell-viability assays are measured as endpoints, so you only get part of the story," explains Peter Banks, scientific director of BioTek Instruments. "Whereas cell proliferation is all about cell growth, and that doesn't happen in a short period of time—depending on the types of cells you're looking at, you won't see any sort of noticeable effect until after several hours—so cell-proliferation assays should be much more of a kinetic look at what cells do."

Tried and true reagents like trypan blue selectively stain cells without compromising membrane integrity, and thus "trypan blue exclusion" is commonly used as a crude mark of viability. This is the basis of low-throughput counting using a hemocytometer, as well as many commercial cell counters.

But researchers often require more than just an intact membrane to consider a cell viable. Metabolic activity, for example, can be assayed by the reduction of tetrazolium salts commonly known as MTT, MTS and XTT, as well as water-soluble tetrazoliums (WSTs), to colored formazan compounds that can be measured quantitatively by absorbance on a plate reader. These salts differ from one another in various respects, such as solubility and cell permeability, color

and solubility of the resulting product and cytotoxicity, leading to a variety of protocols and formulations offered by a host of vendors.

The soluble resazurin—best known as alamarBlue® (provided by Bio-Rad, Thermo Fisher Scientific and many other vendors) can be used like tetrazolium salts, in this case being reduced by the cellular machinery to a soluble pink product, resofurin. And because resofurin is fluorescent, its appearance can be measured much more sensitively by fluorescence reading. Similarly, Promega's CellTiter-Fluor™ assay leverages a cell's constitutive protease activity to turn a cell-permeant, fluorogenic substrate into a fluorescent product.

The most common viability assays in high-throughput screening (HTS) make use of the fact that compromised cells are rapidly depleted of ATP. ATP is required as a cofactor in firefly luciferase's catalysis of luciferin to generate light. Following cell lysis, the sensitive, rapid reaction can be read with a luminometer [1].

Some researchers prefer to assess viability with a two-color "live/dead" assay , for example combining a membrane-permeable esterase substrate (such as calcein-AM, the esterified form of which is unable to leave the cell) with a membrane-impermeant nuclear stain (such as propidium iodide), both of which are virtually nonfluorescent but become fluorescent when they interact with their cellular targets.

Get real

Viability assays give a snapshot of the number, and perhaps the percentage, of cells in a culture that are functional.

But that's not always enough, points out Yuan-Shan Zhu, associate professor and director of the Clinical and Translational Science Center (CTSC) Core Laboratory at Weill Cornell Medical College: "When you study cancer cells, you study proliferation." A culture with twice the signal of another may have doubled relative to that other culture, but "not necessarily—DNA synthesis and metabolic activity are two different criteria."

Nonetheless, endpoint viability assays using sequential time points are often used as surrogates for proliferation, points out Banks, as is evident from how many vendors market such assays. But "what [is] much more useful is just to monitor your cells over time instead of taking readings of different cells."

This can be done in many ways—for example, by using nontoxic reagents such as Promega's RealTime-Glo™ Cell Viability Assay to monitor cell viability over hours or days. This generates a "luminescent signal from live cells only, proportional to the number of live cells," notes Terry Riss, global strategic manager, cell health, at Promega Corp.

Reporter constructs like green fluorescent protein (GFP) are often used to track proliferation and transfection efficiency, with the assumption that GFP expression (and therefore fluorescence) is proportional to cell number (and therefore proliferation).

DNA synthesis itself can be monitored, either as a series of snapshots or in real time, with reagents such as nucleoside analogs that become incorporated into nascent DNA. Radioactive thymidine has largely fallen out of favor; its use has been replaced by protocols involving other analogs, such as bromodeoxyuridine (BrdU, typically imaged with fluorescently labeled antibodies) and 5-ethynyl-2'-deoxyuridine (EdU, used with click chemistry).

Immunologists have for many years labeled cells with carboxyfluorescein succinimidyl ester (CFSE) and its relatives to measure proliferation. The dye enters enter cells and covalently attaches to protein-based amines. As the cell divides, it passes the dye to its daughters, meaning that each successive generation is half as bright as its mother. About seven or either generations can be distinguished by flow cytometry.

Banks thinks it's best to "just watch the cells proliferate."

This can be done using phase contrast imaging, as is the case with Essen Bioscience's IncuCyte ZOOM® in-incubator digital microscope and analysis system.

Meanwhile, BioTek's Cytation 5 Cell Imaging Multi-Mode Reader will introduce a high-contrast brightfield analysis capability this month that "allows us to do cell counting and also percent confluence—we can get really definitive measurements of how your cells are growing without perturbing them," notes Banks.

Keep them happy

Imaging cytometers, high-content analysis workstations and microscopes fitted with environmental chambers all enable live imaging in a constant environment without perturbing the cells over time. Yet when leading up to and running long-term assays—even endpoint assays—giving the cells an environment that more closely mimics the in vivo environment may enable a more physiological assessment of how cells would, say, proliferate in the stem cell niche under the test conditions, points out James Lim, chief scientific officer at Xcell Biosciences. Xcell's desktop-printer-sized Avatar™ System allows for precise control of both oxygen and pressure. "We're able to mimic the range of pressure conditions found all the way from the circulation to the bone marrow to the fluid pressures found within the brain," he says.

Careful selection

There are many options available to researchers who are interested in performing cell-based assays. It is important to understand that there isn't a single experimental solution, and often multiple assays may need to be tested to determine the most appropriate test for the cells you are working with. The assays are not interchangeable, and some assays may be toxic to the cells or not well suited for kinetic analysis. Certain assays are more compatible with adherent vs. suspension cells. Some analyses lend themselves best to plate readers, imagers, microscopes or flow (keeping in mind that "any type of cells can be run on a flow cytometer—people take tumor tissue, transplant tissue and dissociate it and run it through the machine," says Jeffery Boyd, director of the University of Southern California Stem Cell Flow Cytometry Core Facility). Although cost will always be an important consideration when selecting a cell-based assay, researchers should also balance this against factors such as assay sensitivity, throughput, amenability to automation, detection mode and assay format. In any case, there is likely an option, or possibly two, that suit your needs. May your cells live long and proliferate.

Reference

[1] Riss, TL, et al., Cell Viability Assays (chapter), "Assay Guidance Manual" (e-book), published May 1, 2013; updated July 1, 2016.

Image: ShutterStock Images