

Tools for visualizing and quantifying neuronal cell health

Tools that allow simple and rapid visualization and quantification of the fitness of neuronal cells are essential for monitoring the effects of a variety of factors, including biological modifiers, neural cell culture conditions, drug compounds, and environmental neurotoxicants. Highlighted below are five particularly useful assays, each of which measures a different parameter of cell health (Table 1). Moreover, because these assays are nondestructive (i.e., no lysis required), they can be easily combined with additional readouts to provide a more complete multiparametric assessment of neuronal cell health.

Reduced cellular metabolic activity is often a highly sensitive early indicator of failing cell health that precedes the loss of cell membrane integrity. The **PrestoBlue® Cell Viability Reagent** facilitates measuring the metabolic activity of cells via a single addition step of an optimized formulation of the widely utilized reduction substrate resazurin (Figure 1A). This cell-permeant compound undergoes a large color change within the reducing environment of live cells, going from a nonfluorescent blue substrate to a highly fluorescent red product that is secreted into the surrounding medium. This dramatic color change can be readily visualized by eye and quantified using instruments that detect fluorescence or absorbance. Much faster than other resazurin-based solutions, which typically require up to several hours of incubation time, the change in fluorescence with PrestoBlue® reagent can be observed in as little as 10 minutes, depending on the cell type and number of cells being measured.

Abundant in live cells, intracellular esterase activity is quickly lost as cells die and rupture. The acetomethoxy derivate of calcein, **Calcein AM**, is a cell-permeant dye that is essentially nonfluorescent until it enters the cell, where it becomes hydrolyzed into a bright green-fluorescent product that remains trapped within the cell. Thus, Calcein AM can serve as a useful measure of cell viability via its dual mechanistic dependency on enzymatic esterase activity, which is required to activate its fluorescence, as well as cell membrane integrity, which is required for its intracellular retention (Figures 1B and 1E). In general, Calcein AM can be directly applied to cells and incubated for 10–30 minutes with good results. To minimize potential background fluorescence arising from extracellular esterase activity commonly found in serum-containing medium, it is advisable that a medium removal and wash step also be incorporated into the workflow where practical.

Since cellular DNA content is tightly controlled, estimates of cell numbers based on DNA are usually very accurate. The **CyQUANT® Direct Cell Proliferation Assay** is designed to measure the DNA content from live cells whose membranes have not been disrupted (Figures 1C and 1F). This is done by combining a cell-permeant DNA-binding dye with a background suppressor dye that obscures the staining of dead cells with compromised plasma membranes, resulting in bright green fluorescence of only the intact live cells. Accordingly, the CyQUANT® Direct assay has proven to be very useful for monitoring cell proliferation as well as assessing cell viability and cytotoxicity effects. Once the two-component working solution is prepared, it is simply added to the cells, and results can be visualized and quantified typically within 30–60 minutes.

Table 1. Comparison of selected tools that can be used to measure different aspects of neuronal cell health.

Assay	Cat. No.	Parameter measured	Readout	Assay time	Workflow summary	Instrumentation options	Multiplex options
PrestoBlue® Cell Viability Reagent	A13261	Metabolic activity/reducing power of live cells	Blue (non-fluorescent) to red (fluorescent) color change	10 min–2 hr	1. Add to cells 2. Incubate 3. Measure	Absorbance, fluorescence plate reader (bottom- or top-read), spectrophotometer	CyQUANT® Direct assay or Calcein AM
Calcein AM*	C3099	Intracellular esterase activity (indicates cell membrane integrity)	Green fluorescence	10–30 min	1. Wash cells (optional) 2. Add to cells 3. Incubate 4. Measure	Imaging/microscopy, fluorescence plate reader (bottom- or top-read)	PrestoBlue™ Cell Viability Reagent or Ethidium Homodimer-1
CyQUANT® Direct Cell Proliferation Assay	C35011	Binds DNA in live cells (indicates cell membrane integrity)	Green fluorescence	30–60 min	1. Add to cells 2. Incubate 3. Measure	Imaging/microscopy, fluorescence plate reader (bottom-read only)	PrestoBlue™ Cell Viability Reagent or Ethidium Homodimer-1
Ethidium Homodimer-1* (EthD-1)	E1169	Binds DNA in dead cells (indicates loss of cell membrane integrity)	Red fluorescence	30 min	1. Add to cells 2. Incubate 3. Measure	Imaging/microscopy, fluorescence plate reader (bottom- or top-read)	CyQUANT® Direct assay or Calcein AM
Neurite Outgrowth Staining Kit	A15001	Stains outer membrane surfaces, measures intracellular esterase activity	Orange fluorescence and green fluorescence	<30 min	1. Replace medium with stain 2. Incubate 3. Replace stain with background suppressor 4. Measure	Imaging/microscopy, fluorescence plate reader (bottom-read only)	Already a multiplexed kit

*Calcein AM and EthD-1 are also available together as the LIVE/DEAD® Viability/Cytotoxicity Kit (Cat. No. L3224).

Whereas the CyQUANT® Direct assay stains only live cells without compromised cell membranes, another useful DNA stain, **Ethidium Homodimer-1** (EthD-1), stains dead cells and leaves live cells unstained (Figures 1D and 1F). By itself, EthD-1 is a weak fluorescent molecule that is too large and positively charged to cross the cell membrane of live cells. When the membrane integrity of dying or dead cells is compromised, EthD-1 can enter the cell where, upon binding to nucleic acids, it undergoes a ~40-fold enhancement of bright red fluorescence. Thus, EthD-1 can serve as a useful gain-of-signal stain to positively mark the dead cell population, and is complementary to the commonly employed loss of viability approaches. Following a single addition step, cells are typically incubated for 30 minutes prior to imaging and/or measuring the red fluorescence of the dead cells.

Neurite outgrowth is an important morphological phenotype of neuronal cells that correlates with their function and cell health. Unlike conventional neurite immunostaining approaches that are tedious and time-consuming to perform, the dual-color **Neurite Outgrowth Staining Kit** was developed using a select combination of fluorescent and background suppression dyes that allows simple, rapid visualization and relative quantification of neurite outgrowth along with cell viability (Figure 2). Neurite outgrowth is monitored via bright orange-fluorescent staining of outer cell membrane surfaces. Neuronal cell viability is assessed simultaneously via the use of a cell-permeant viability indicator dye that is converted by live cells to a form that emits green fluorescence. A typical workflow using this kit requires less than 30 minutes and a few simple steps prior to taking a measurement.

To find more information about neuronal cell health assays, visit lifetechnologies.com/neurocellhealth

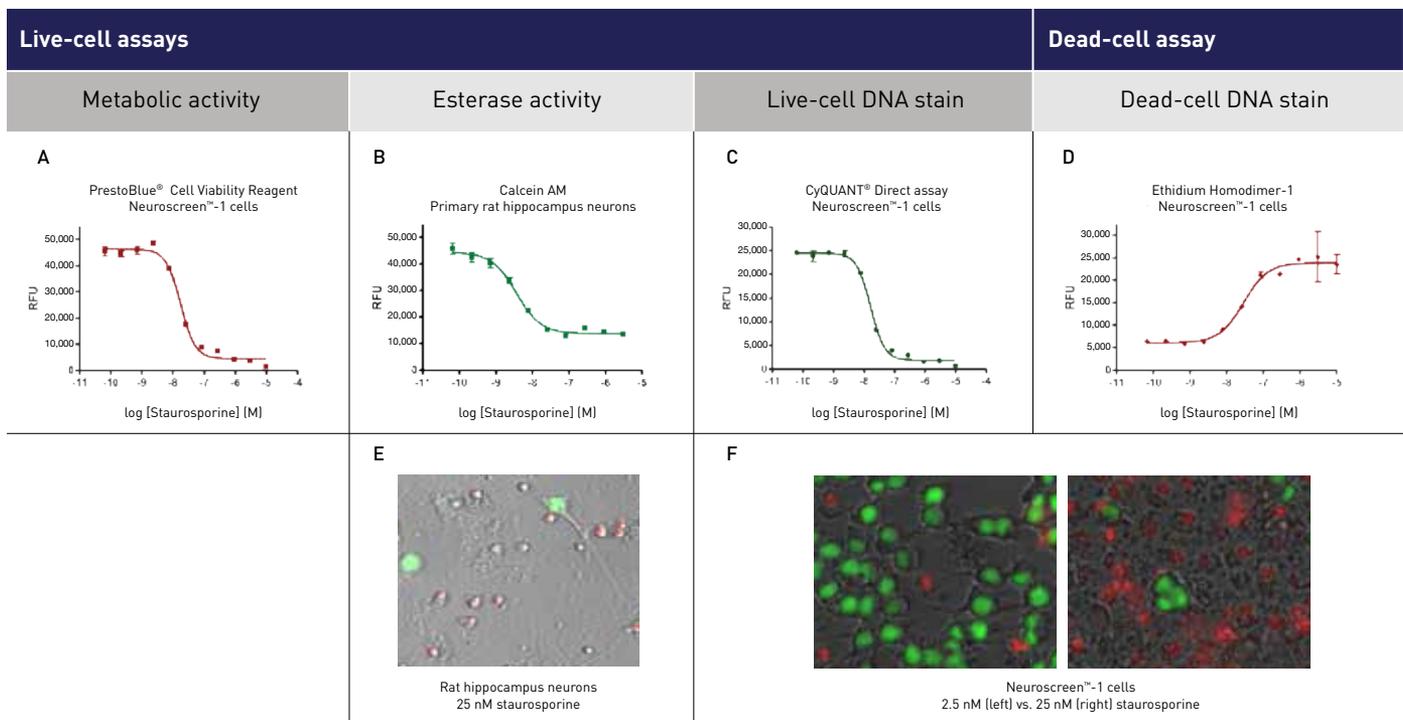


Figure 1. Visualizing and quantifying neuronal cell health. PC12-derived Neuroscreen™-1 cells (**A, C, D, F**) or rat hippocampus neurons (**B, E**) were treated with a serial dilution of the cytotoxic compound staurosporine, and loss of cell viability was measured using a fluorescence plate reader at 24–48 hr post-treatment. Also shown are multiplexing image examples, in which overlaid onto the bright-field view is red-fluorescent staining of dead cells using Ethidium Homodimer-1, combined with green-fluorescent staining of live cells using either Calcein AM (**E**) or the CyQUANT® Direct assay (**F**).

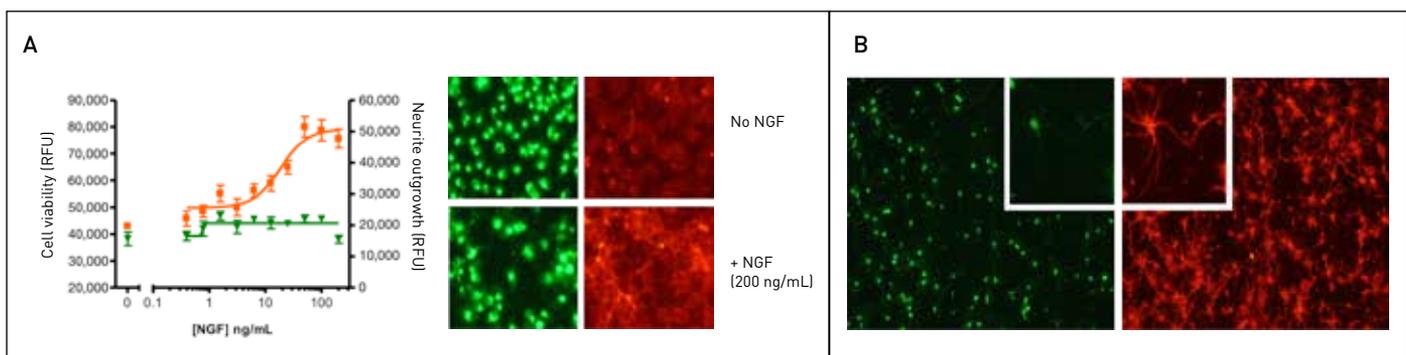


Figure 2. Visualizing and quantifying cell viability and neurite outgrowth using the dual-color Neurite Outgrowth Staining Kit.

(A) PC12-derived Neuroscreen™-1 cells were treated with a serial dilution of nerve growth factor (NGF) for 4 days prior to measuring relative cell viability and neurite outgrowth using a plate reader (left panel) and taking images of representative wells (right panel). The Neurite Outgrowth Staining Kit simultaneously measures cell viability (green fluorescence), which was unchanged in this experiment, and relative neurite outgrowth (orange fluorescence), which increased in an NGF dose-dependent manner.

(B) Cryopreserved primary rat cortex neurons were plated and grown in Neurobasal® medium (Cat. No. 21103-049) supplemented with B-27® Serum Free Supplement (Cat. No. 17504-044) for 7 days prior to staining with the Neurite Outgrowth Staining Kit. Left panel: image of cells stained with the kit's green-fluorescent cell viability indicator, which primarily stains the cell bodies of live cells. Right panel: image of cells in the same field of view stained with the kit's orange-fluorescent cell membrane stain, which stains the membranes of neurite extensions in addition to cell bodies.

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