Neurite Outgrowth Staining Kit, Catalog no. A15001

Kit components	Volume	Storage and handling
Cell Membrane Stain (1000X)	100 µL	-20 °C, long-term (≥6 months); room temperature up to 60 days
Cell Viability Indicator (1000X)	100 µL	-20 °C, long-term (≥6 months); room temperature up to 60 days
Background Suppression Dye (100X)	2.5 mL	-20 °C, long-term (≥6 months); room temperature up to 60 days; use sterile technique to avoid contamination
Number of assays: Sufficient reagent is supplied for: 250 assays* (24-well format) or 1,000 assays* (96-well format) *calculations assume 400 µL and 100 µL volumes used for 24-well and 96-well formats, respectively.		
Approximate fluorescence excitation/emission maxima: 555 nm/565 nm (TRITC/Cy3 filter-compatible) for the Cell Membrane Stain, 495 nm/515 nm (FITC/fluorescein filter-compatible) for the Cell Viability Indicator.		

I. Product Overview

How does the Neurite Outgrowth Staining Kit work?

Neurite outgrowth is an important morphological phenotype of neural cells that correlates with their function and cell health. The Molecular Probes® **Neurite Outgrowth Staining Kit** provides a two-color fluorescence assay that enables simple, rapid visualization and quantification of neurite outgrowth and cell viability in the same sample. Relative neurite outgrowth is monitored via bright orange staining of outer cell membrane surfaces – changes in the number or length of neurite processes extending from the neural cell body directly correlate with the relative amount of cell membrane surface area that is available to be stained. Cell viability is simultaneously measured via the use of a cell-permeant viability indicator dye, which is converted by the intracellular esterase activity of living cells to emit green fluorescence primarily in the neural cell bodies. Inclusion of a background suppressor reagent minimizes fluorescence background interference arising from the staining of non-cell surfaces. Together this optimized combination of staining reagents allows for measuring neurite outgrowth and cell viability in the same sample.

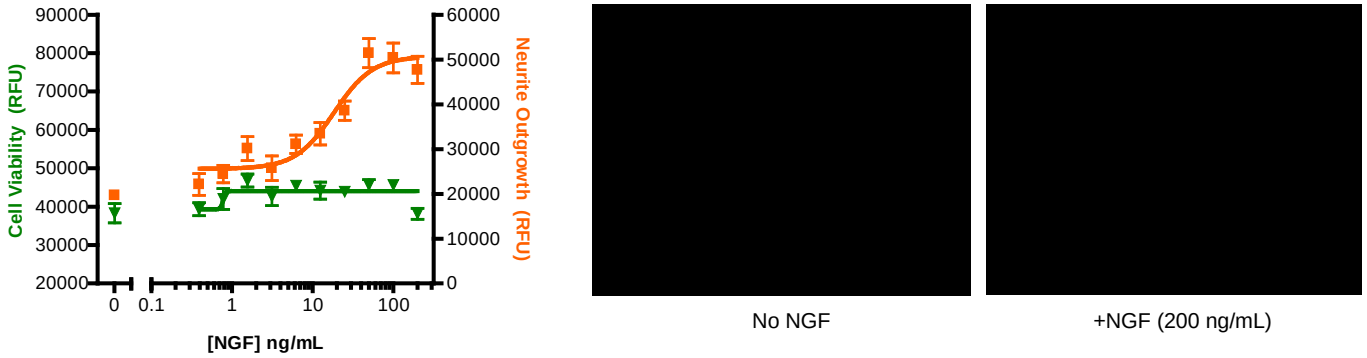


Figure 1. Example data using the Neurite Outgrowth Staining Kit. PC12-derived Neuroscreen™-1 cells were treated with a serial dilution of nerve growth factor (NGF) for 4 days prior to measuring relative cell viability and neurite outgrowth using a plate reader (left panel) and taking images of representative wells (right panels). 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.

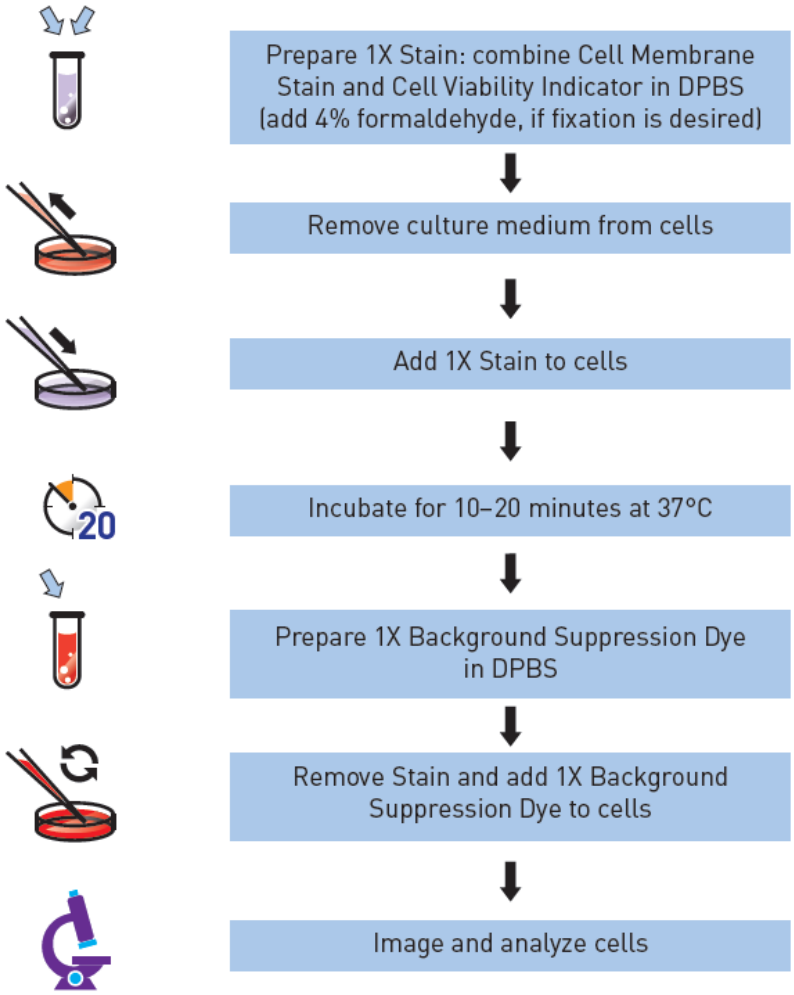
How does the Neurite Outgrowth Staining Kit compare to other methods for measuring neurite outgrowth or neural cell viability?

Table 1. Comparison of select methods for measuring neurite outgrowth and/or neural cell health.

	Neurite Outgrowth Staining Kit	Immunostaining (e.g., βIII-tubulin)	LIVE/DEAD® Viability/Cytotoxicity Kit	PrestoBlue® Cell Viability Reagent
Measure neurite outgrowth	**Y**	**Y**	**N**	**N**
Measure cell viability	**Y**	**N**	**Y**	**Y**
Time to result	**15 – 30 min**	**3 – 4 hr**	**30 – 60 min**	**10 min – 2 hr**
Ease of use	**+++**	**+**	**++**	**++++**
Specific to neurons	**N**	**Y**	**N**	**N**
Live-cell assay	**Y**	**N**	**N**	**Y**
Cells can be fixed	**Y**	**Y**	**N**	**N**
Imaging/microscopy-compatible	**Y**	**Y**	**Y**	**N**
Microplate reader-compatible	**Y**	**N**	**Y**	**Y**

What is a typical assay workflow using the Neurite Outgrowth Staining Kit?

A typical workflow using this kit requires a total of 15 – 30 minutes and a few pipette steps prior to taking a measurement.



What controls should I include in my experiment?

The particular set of controls required may depend upon your experimental objective, but typically the following controls are included particularly if quantification of relative neurite outgrowth and cell viability is to be performed:

1. **Cell-free control** – control containing medium without any cells, but which undergoes the same staining manipulations as the cell-containing samples. This cell-free control is useful for checking the fluorescence "background" of the experimental setup (e.g., glass or plastic-ware fluorescence background, effects of any carryover of medium or stain during the pipette steps). The cell-free background control data can be subtracted from the results obtained for the cell-containing assay wells.

2. **Positive and negative controls** – for comparing relative neurite outgrowth, it is important to include controls in which little or no neurite outgrowth is expected and/or controls in which maximal neurite outgrowth is expected. Similarly, for comparing relative cell viability, control conditions that elicit a low and high degree of cell viability can be included in the experiment.

3. **Cell treatment controls** – as applicable, control cells treated with the same vehicle (e.g., DMSO, PBS) or medium that is used for preparing any compound or biological treatment that may be tested. Cell treatment controls often serve a dual purpose as negative controls (i.e., have no effect on cell viability or neurite outgrowth).

II. Storage and Handling

What are the recommended storage and handling requirements for the Neurite Outgrowth Staining Kit components?

The performance of the Neurite Outgrowth Staining Kit is guaranteed for 6 months from the date of purchase if stored and handled properly, with a recommended storage temperature of -20 °C for maximal reagent stability. The individual dyes have been tested and shown to withstand multiple freeze-thaws without any adverse effects on stability or performance.

For convenience, the Neurite Outgrowth Staining Kit components can also be stored at room temperature for up to 60 days with little or no effect on their stability. When handling, use sterile technique to avoid introducing contamination.

Table 2. Storage and handling guidelines for the Neurite Outgrowth Staining Kit.

Kit component	Storage and handling
Cell Membrane Stain	-20 °C, long-term (≥6 months); room temperature up to 60 days
Cell Viability Indicator	-20 °C, long-term (≥6 months); room temperature up to 60 days
Background Suppression Dye	-20 °C, long-term (≥6 months); room temperature up to 60 days; use sterile technique to avoid contamination

How light sensitive are the Neurite Outgrowth Staining Kit components?

Each of the dyes provided in the Neurite Outgrowth Staining Kit has been tested for light sensitivity and shown to be relatively stable to prolonged light exposure and resistant to photo-bleaching. Short-term exposure to ambient lighting or microscopy light had little or no detectable effect on performance. Nevertheless, as with any dye, it is advisable to minimize prolonged light exposure, particularly to direct sunlight.

What if I accidentally left the Neurite Outgrowth Staining Kit at room temperature overnight?

The 1000X dye stocks provided in the Neurite Outgrowth Staining Kit are stable at room temperature for up to 60 days. However, once the dyes have been diluted in buffer to prepare a 1X working stain solution, for best performance the 1X stain solution should be used immediately (i.e., within ~15 – 30 minutes following dilution) and any remaining solution should be discarded.

Once I've prepared the 1X working stain solution, can I save it for later use?

No – once the dyes have been diluted in buffer to prepare a 1X working stain solution, for best performance the 1X stain solution should be used immediately (i.e., within ~15 – 30 minutes following dilution) and any remaining solution should be discarded. Once it is diluted, the Cell Viability Indicator is susceptible to hydrolysis over time that can render it unusable. Following dilution, the Cell Membrane Stain is a relatively sticky dye that in addition to cells can also readily absorb to and adhere to a variety of surfaces, including tubes and pipette tips. Accordingly, for consistent staining it is important to use only freshly prepared stain, to mix well, and to minimize the amount of time the 1X working stain is allowed to stand before it is applied to cells.

III. Usage

Can the Neurite Outgrowth Staining Kit also be used to stain non neuronal cell types?

Yes – because the dyes used in this kit are not specific to neuronal cell types, almost any animal cell type can be used with the Neurite Outgrowth Staining Kit to stain cell membrane surfaces and assess cell viability. However, the stain concentration and/or incubation time may need to be optimized for different cell types. Notably, like neural cells, many glial cells (e.g., astrocytes and oligodendrocytes) also exhibit membrane processes or projections that present important morphological features that can be measured with the Neurite Outgrowth Staining Kit.

Can the individual dyes within the Neurite Outgrowth Staining Kit be used separately?

Yes – to enable user flexibility and maximize reagent stability the dyes used in the Neurite Outgrowth Staining Kit are provided as separate stocks and can therefore be used separately as desired. For example, some researchers may prefer to stain the nuclei of their cells with a DNA binding dye (e.g., DAPI or Hoechst) and may omit using the green-fluorescent Cell Viability Indicator and instead use only the orange-fluorescent Cell Membrane Stain.

Can the Neurite Outgrowth Staining Kit be used with live cells or fixed cells?

Yes – protocols for live-cell staining as well as fixed-cell staining are provided with the kit or are available online.

After live-cell staining is performed, can the cells continue to be cultured and later re-stained?

In general, yes, although certain cell types or culture conditions may be less suitable for continued culturing and re-staining after the initial staining procedure. Testing performed during the development of the Neurite Outgrowth Staining Kit indicated that the dyes used in this kit are relatively nontoxic to cells, particularly when the staining incubation time is minimized and the cells are restored to fresh medium as soon as possible. Utilization of buffers or specialized medium (e.g., Live Cell Imaging Solution, catalog # A14291DJ) that promote cell health during staining procedures may also help facilitate post-staining cell viability. Furthermore, while some modest residual staining may linger for a day or two, it was shown that cells could be effectively re-stained as early as 24 hours following initial staining.

IV. Fluorescence imaging and plate reader settings

What are the recommended fluorescence imaging settings for use with the Neurite Outgrowth Staining Kit?

Standard FITC or fluorescein filter settings work well for the green-fluorescent Cell Viability Indicator whereas standard TRITC or Cy3 filter settings are suitable for the orange-fluorescent Cell Membrane Stain.

Table 3. Approximate excitation and emission maxima

Dye	Excitation (nm)	Emission (nm)
Cell Viability Indicator	495	515
Cell Membrane Stain	555	565

What are the recommended microplate reader settings for use with the Neurite Outgrowth Staining Kit?

Quantification of relative fluorescence can be performed using a monochromator (see Table 4) or filter-based (see Table 5) fluorescence plate reader. The settings guidance provided here may need to be adjusted for your particular instrument keeping in mind the approximate excitation and emission maxima of each dye (see Table 3). Note that it is advisable to include a cell-free control for assessing fluorescence background.

Important: only bottom-read mode should be used with cells stained in a clear-bottom microplate. Take care to keep the plate bottom free from fingerprints, dust, and lint (e.g., wipe clean or use compressed air to remove dust).

Table 4. Plate reader setup guidance for monochromator-based instruments

Dye	Excitation (nm)	Emission (nm)	Bandwidth (nm)
Cell Viability Indicator	483	525	12
Cell Membrane Stain	554	567	5

Table 5. Plate reader setup guidance for filter-based instruments

Dye	Excitation (nm)	Emission (nm)	Bandwidth (nm)	Dichroic mirror
Cell Viability Indicator	480	520 or 535	25 or 30	510 or 50:50 band splitter
Cell Membrane Stain	531 or 535	579 or 590	25 or 20	555 or 560

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