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Neurite Outgrowth Staining Kit

Catalog no. A15001

Table 1 Contents and storage

Kit Component	Amount	Storage*	Shelf Life
Cell Membrane Stain (1000X) in DMSO	100 µL	≤–20°C	When stored as directed, the product is stable for at least 6 months.
Cell Viability Indicator (1000X) in DMSO	100 µL		
Background Suppression Dye (100X)	2.5 mL		
Shipping: The product is shipped at room temperature.			

* Background Suppression Dye may be stored at room temperature.

Introduction

Neurite outgrowth is an important morphological phenotype of neuronal cells that correlates with their function and cell health. The dual-color Neurite Outgrowth Stain has been developed using a select combination of fluorescent and background suppression dyes that allow for simple, rapid visualization and relative quantification of neurite outgrowth along with neuronal cell viability in the same sample. Neurite outgrowth is monitored via bright orange-red staining of outer cell membrane surfaces. Neuronal cell health is assessed simultaneously by means of a cell-permeable viability indicator dye that is converted by live cells to emit green fluorescence (see Figure 1, page 2).

The Neurite Outgrowth Staining Kit can be used:

- to visualize and quantify relative neurite outgrowth.
- to visualize and quantify cell viability from the same sample. Note that the cell membrane dye stains both live and dead cells, whereas the cell viability indicator only stains live cells.
- with live or fixed (but not permeabilized) cells; live cells can be re-stained as early as 24 hours later.

The Neurite Outgrowth Staining Kit is not recommended for:

- discriminating between neuronal and non-neuronal cell types (i.e., the dyes used in this kit are not specific for neuronal cells, but will stain all cell types).
- use with cell permeabilization protocols.

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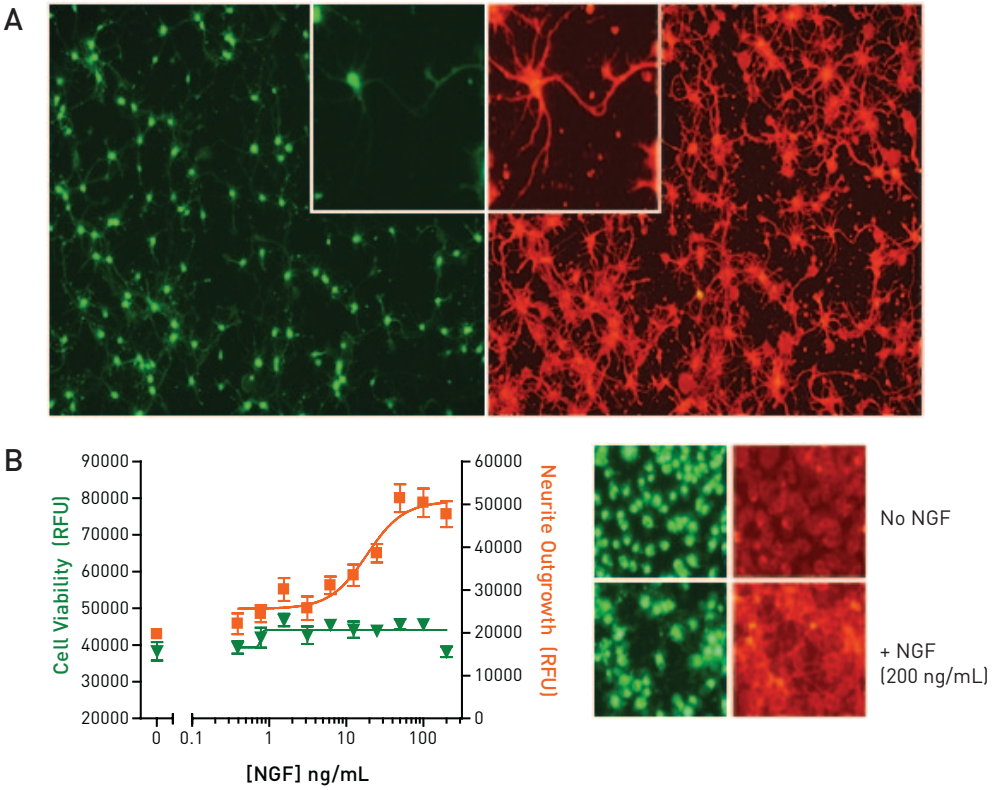
Figure 1 Example results using the Neurite Outgrowth Staining Kit.

[A] Cryopreserved primary rat cortex neurons (Cat. no. A10840-02) were thawed and plated in 96-well format and grown in Neurobasal® medium (Cat. no. 21103-049) supplemented with B-27® Serum-Free Supplement (Cat. no. 17504-044) and 0.5 mM GlutaMAX™ (Cat. no. 35050) for 7 days prior to live-cell staining with the Neurite Outgrowth Staining Kit. Left, representative image of the green fluorescent Cell Viability Indicator, which primarily stains the cell bodies of live cells. Right, image of the orange fluorescent Cell Membrane Stain in the same field of view, which stains the membranes of neurite extensions in addition to cell bodies.

[B] Neuroscreen™-1 cells, a PC12 subclone, were plated in 96-well format and treated with a dilution series of nerve growth factor (NGF) for 4 days prior to assaying with the fix and stain protocol. Left, relative fluorescence for cell viability (green) and neurite outgrowth (orange) was measured using a fluorescence plate reader (n = 4 replicates ± S.E.). Right, representative images of the cells with or without NGF treatment.



Before Starting

Materials Required but Not Provided

- A physiologically relevant buffer, e.g., Dulbecco's Phosphate Buffered Saline containing calcium andmagnesium (DPBS, Cat. no. 14287-080) or Hank's Balanced Salt Solution containing calcium and magnesium (HBSS, Cat. no. 14025-076)
- *Optional:* 4% formaldehyde in buffer for fixation

Caution

DMSO is hazardous; avoid contact with skin and eyes and do not swallow. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials.

Preparing 1X Working Stain Solution

1.1 Thaw the reagents at room temperature and ensure that they are completely in solution before proceeding. Note that the Background Suppression Dye can also be stored at room temperature.

1.2 To prepare a 1X working Stain Solution (see Table 2, below), combine the Cell Viability Indicator and the Cell Membrane Stain in Dulbecco's Phosphate-Buffered Saline containing calcium and magnesium (Cat. no. 14287-080) or a similar buffered solution. Mix well. Prepare enough 1X stain to completely cover each well or dish to be assayed.

Table 2 Volumes for preparing a 1X working Stain Solution

Culture Format (plate/dish)	Cell Viability Indicator (1000X)*	Cell Membrane Stain (1000X)*†	Buffer	Application Volume (per well/dish)
96-well plate	10 µL	10 µL	10 mL	100 µL
48-well plate	10 µL	10 µL	10 mL	200 µL
24-well plate	10 µL	10 µL	10 mL	400 µL
12-well plate	10 µL	10 µL	10 mL	800 µL
6-well plate	10 µL	10 µL	10 mL	1.5 mL
35-mm dish (10 cm^2)	1.5 µL	1.5 µL	1.5 mL	1.5 mL
60-mm dish (20 cm^2)	3 µL	3 µL	3 mL	3 mL
100-mm dish (60 cm^2)	9 µL	9 µL	9 mL	9 mL

* Approximate stock concentration. Depending upon the sensitivity of your imaging and/or plate reader instrumentation, it may be necessary to adjust the concentration of either one or both dyes for more optimal performance (e.g., test 2-fold more or less dye in the same volume of buffer).
† Once it is diluted, 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.

Experimental Protocols

The following two protocols are provided as guidance, and further optimization may be required depending on your specific circumstances. For comparing relative neurite outgrowth, it is recommended that appropriate controls are included in the experiment (e.g., control cells with little or no neurite outgrowth). For plate reader quantification, it is advisable to also have a cell-free control for subtracting the fluorescence background.

Live-Cell Staining Protocol

2.1 Prepare 1X working Stain Solution as described above. Make sure that you have enough 1X stain to completely cover each well or dish to be assayed.

2.2 Remove the medium from the cultures to be stained.

Optional: Rinse the cultures to be stained with PBS and aspirate.

2.3 Apply an appropriate volume (see Table 2, above, for guidance on application volume) of the 1X working Stain Solution to each well or dish to be assayed.

2.4 Incubate the cultures for 10–20 minutes at room temperature or 37°C.

2.5 While incubating, prepare a 1X working solution of the Background Suppression Dye by diluting it 100-fold (e.g., 10 µL per 1 mL of buffer) into Dulbecco's Phosphate-Buffered Saline containing calcium and magnesium (Cat. no. 14287-080) or a similar buffered solution. Mix well. Prepare enough 1X Background Suppression Dye to completely cover each well or dish to be assayed.

2.6 Following the staining incubation, remove the stain and discard it.

Optional: Rinse the cultures with PBS and aspirate.

2.7 Apply an appropriate volume (see Table 2, page 3, for guidance on application volume) of the 1X working Background Suppression Dye to each well or dish to be assayed.

2.8 Visualize the cells (see **Fluorescence Imaging Guidance**, page 5) and/or perform fluorescence plate reader quantification (see **Fluorescence Plate Reader Guidance**, page 5).

2.9 *Optional:* Following visualization and/or quantification, replace the Background Suppression Dye with fresh cell culture medium and continue to maintain the culture. Stained cells may be re-stained as early as 24 hours after the initial staining.

Fix and Stain Protocol

3.1 Prepare a fresh 1X working Fix/Stain Solution (see Table 2, page 3) by combining the Cell Viability Indicator and Cell Membrane Stain in buffer containing 4% formaldehyde. Mix well. Prepare enough 1X stain to completely cover each well or dish to be assayed.

3.2 Remove the medium from the cultures to be stained.

Optional: Rinse the cultures to be stained with PBS and aspirate.

3.3 Apply an appropriate volume (see Table 2, page 3, for guidance on application volume) of the 1X working Fix/Stain Solution to each well or dish to be assayed.

3.4 Incubate the cultures for 10–20 minutes at room temperature or 37°C.

3.5 While incubating, prepare a 1X working solution of the Background Suppression Dye by diluting it 100-fold (e.g., 10 µL per 1 mL of buffer) into Dulbecco's Phosphate-Buffered Saline containing calcium and magnesium (Cat. no. 14287-080) or a similar buffered solution. Mix well. Prepare enough 1X Background Suppression Dye to completely cover each well or dish to be assayed.

3.6 Following the staining incubation, remove the stain and discard it.

Optional: Rinse the cultures with PBS and aspirate.

3.7 Apply an appropriate volume (see Table 2, page 3, for guidance on application volume) of the 1X working Background Suppression Dye to each well or dish to be assayed.

3.8 Visualize the cells (see **Fluorescence Imaging Guidance**, page 5) and/or perform fluorescence plate reader quantification (see **Fluorescence Plate Reader Guidance**, page 5).

Appendix

Fluorescence Imaging Guidance

Image the samples immediately using appropriate fluorescence filter settings (see Table 3, below). Standard FITC or fluorescein filter settings work well for the green Cell Viability Indicator, and standard TRITC or Cy3 filter settings are suitable for the 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

Fluorescence Plate Reader Guidance

Quantification of relative fluorescence can be performed using a monochromator (see Table 4, below) or filter-based (see Table 5, below) 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, above). Note that it is advisable to include a cell-free control for assessing fluorescence background, which can be subtracted from the values obtained for the cell-containing wells.

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 (nm)
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

Purchaser Notification

Corporate Headquarters
5791 Van Allen Way
Carlsbad, CA 92008
USA
Phone: +1 760 603 7200
Fax: +1 760 602 6500
Email: techsupport@lifetech.com

European Headquarters
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF
UK
Phone: +44 141 814 6100
Toll-Free Phone: 0800 269 210
Toll-Free Tech: 0800 838 380
Fax: +44 141 814 6260
Tech Fax: +44 141 814 6117
Email: euroinfo@invitrogen.com
Email Tech: eurotech@invitrogen.com

Japanese Headquarters
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Japan
Phone: +81 3 5730 6509
Fax: +81 3 5730 6519
Email: jpinfo@invitrogen.com

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