

IncuCyte® NeuroLight Orange Lentivirus—Synapsin Promoter

Catalog number: 4758

Contents

- 1x vial of IncuCyte® NeuroLight Orange Lentivirus - Synapsin promoter (0.45 mL/vial)

Storage and stability

The NeuroLight Orange Reagent should be stored at -80°C . Avoid repeated freeze-thaw cycles. Lentivirus is stable for at least 3 months from date of receipt when stored at -80°C .

Test size

Material supplied is sufficient for 1 x 96-well plate.

Product description

The NeuroLight Orange is a lentiviral based live-cell neuronal labeling reagent driven by a synapsin promoter, resulting in the long term expression of orange fluorescent protein (TagRFP) in neuronal cell bodies and neurites. The NeuroLight Orange Reagent ensures highly-efficient, yet non-disruptive labeling of primary or iPSC-derived neurons over days and weeks, and enables the kinetic quantification of neurite length and branching in the presence of astrocytes and other

non-neuronal cell types such as microglia.

The NeuroLight Orange Reagent has been validated for use with the IncuCyte® S3 Live-Cell Analysis System for Neuroscience (Cat. No. 4763) for measurements of neuronal activity and functional connectivity. The IncuCyte S3 for Neuroscience is configured with an Orange/NIR Optical Module. The NeuroLight Orange Reagent is not compatible with IncuCyte instruments configured with a Red/Green Optical Module, e.g. Cat. No. 4647.

Virus Description

3rd generation HIV-based, VSV-G pseudotyped lentiviral particles encoding an orange fluorescent protein (TagRFP).

- Promoter: Synapsin
- Spectral Properties: Ex (max): 555 nm; Em (max): 584 nm

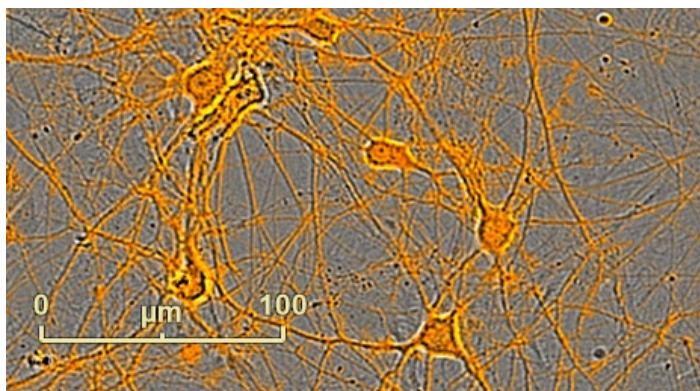
Third generation lentiviral-based vectors are commonly used to transfer genetic

information to cells for gene therapy and/or research purposes. The IncuCyte NeuroLight Orange lentiviral-based reagent has been specially designed to efficiently transduce multiple neuronal cell types with low toxicity. The NeuroLight Orange Reagent encodes an orange fluorescent protein driven off a synapsin promoter to strengthen neuronal expression and minimize non-neuronal crossover. Our extensive validation experiments have shown that expression of this orange fluorescent protein does not negatively alter functional cell biology (e.g. morphology, neurite outgrowth, and neurite branching) of neurons in co-culture with astrocytes. In combination, the IncuCyte S3 for Neuroscience and NeuroLight Orange Reagent provide an integrated solution for kinetically measuring neurite dynamics in vitro.

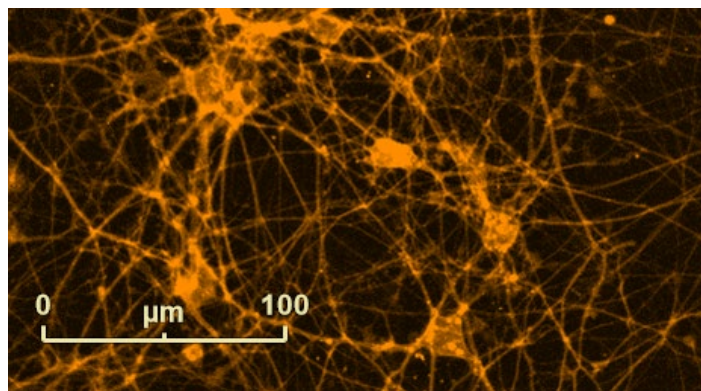
Example data

NOTE: This product is designed for use in a neuronal co-culture assay format. Performance in a mono-culture format has not been validated.

IncuCyte images of the IncuCyte® rCortical Neurons in co-culture with IncuCyte® rAstrocytes.



Phase-contrast/fluorescent blended image showing cortical neurons infected with NeuroLight Orange Reagent. Scale bar: 100 μm .



Fluorescent image of rCortical Neurons expressing NeuroLight Orange Reagent, same field of view as image on left. Scale bar: 100 μm .

Protocols: IncuCyte® NeuroLight Orange Reagent

Materials required but not provided

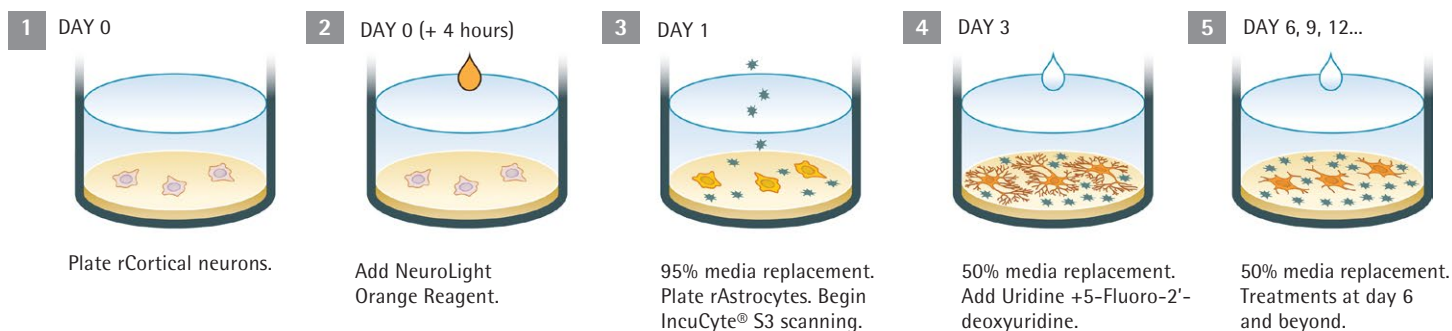
Software

- IncuCyte® S3 Live-Cell Analysis System for Neuroscience (Cat. No. 4763) with IncuCyte® NeuroTrack Software Module (Cat. No. 9600-0010)

Reagents

- 5-Fluoro-2'-deoxyuridine – Sigma Aldrich (Cat. No. F0503)
- Uridine – Sigma Aldrich (Cat. No. U3003)
- Surface coating materials for 96 well plate

Protocol Overview: IncuCyte® NeuroLight Orange Cell Reagent



CRITICAL: Use rigorous aseptic technique at all times.
Only open the culture plate and medium bottles within a tissue culture hood.

Protocol: Neuronal co-culture

- 1) Plate neurons at desired density and on matrix of choice in a 96-well plate and incubate at room temperature for 30 minutes. Place in incubator and allow 2-4 hours for cells to adhere.
 - 2) Add appropriately diluted NeuroLight Orange Reagent to achieve desired concentration. The final well volume should be 200 μ L per well.
Note: Quality control for the IncuCyte NeuroLight Orange Reagent is the ability to efficiently infect IncuCyte rCortical Neurons to express TagRFP, driven off of the synapsin promoter of the IncuCyte NeuroLight Orange Lentivirus, such that a volume of > 3.2 μ L/20,000 neurons results in a neurite length of > 50 mm/mm² in a neurite outgrowth assay (rCortical Neurons/ rAstrocytes co-culture experiment). We recommend performing a volumetric titration from 100-0.14 μ L for each neuronal cell line evaluated. The lowest concentration that results in the highest neurite outgrowth measurement should be selected. Evaluation of neurite dynamics is to be performed on an IncuCyte S3 for Neuroscience.
 - 3) Incubate the 96-well plate at 37° C for 16-24 hours.
 - 4) Remove 190 μ L transduction media and add 140 μ L/well of appropriate neuronal medium.
 - 5) Initiate co-culture by plating 50 μ L of astrocytes on top of the infected neurons. We recommend seeding astrocytes at 15,000 – 20,000 viable cells/well, whether astrocyte suspension is prepared from fresh stocks or cryopreserved cells.
 - 6) Place plate in the incubator, on a microplate tray in the IncuCyte S3 for Neuroscience, to initiate assay.
 - 7) Approximately 48 hours post-plating astrocytes, remove 100 μ L of media from each well and replace with 100 μ L fresh media containing 2x concentrations of 5-Fluoro-2'-deoxyuridine and uridine to a final assay concentration of 8 μ g/mL and 28 μ g/mL, respectively, in order to arrest astrocyte proliferation.
 - 8) Monitor the cultures over the next 5-12 days, performing a 50% media change every third day.
- Transduction efficiencies of 60-70% are typical. In some cases, it may be preferred to use a lower concentration in order to track neurite dynamics in a high density culture.

Related Products:

Catalog # 4760 IncuCyte® NeuroPrime Orange Cell and Reagent Kit
Catalog # 4586 IncuCyte® rAstrocytes

Catalog # 4753 IncuCyte® rCortical Neurons
Catalog # 9600-0010 IncuCyte® NeuroTrack Software Module

Safety Considerations

The backbone of the Lentivirus particles in this system has been modified to improve their safety and minimize their relation to the wild-type, human HIV-1 virus. These modifications include:

- The lentiviral particles are replication-incompetent and only carry the non-oncogenic gene of interest.
- A deletion in the 3' LTR (U3) resulting in "self-inactivation" (SIN) of the Lentivirus after transduction and genomic integration of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). This alteration renders the lentiviral genome incapable of producing package able virus following host integration.
- The envelope is pseudotyped with the VSV-G gene from Vesicular Stomatitis Virus of the HIV-1 envelope (Burns et al., 1993; Emi et al., 1991; Yee et al., 1994).

Replication-defective lentiviral vectors, such as the 3rd generation vector provided in this product, are not known to cause any diseases in humans or animals. However, lentivirus particles still pose some biohazardous risk because they can transduce primary human cells and can integrate into the host cell genome thus posing some risk of insertional mutagenesis. For this reason, **we highly recommend that you treat lentiviral stocks as Biosafety**

Biohazard Note

The NeuroLight Orange lentivirus contain 3rd generation HIV-based, VSV-G lentiviral particles. The lentiviral particles are replication-incompetent and only carry the non-oncogenic gene of interest. Further, a deletion in the 3' LTR (U3) results in "self-inactivation" (SIN) of the Lentivirus after transduction and genomic integration of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey et al., 1998). This alteration renders the lentiviral genome incapable of producing packageable virus following host integration. Finally,

Level 2 (BSL-2, BL-2) organisms and strictly follow all published BL-2 guidelines with proper waste decontamination.

For more information about the BL-2 guidelines and Lentivirus handling, we recommend referring to local documentation based on geography. The Essen BioScience 3rd generation HIV-based lentivirus' meet BL-2 requirements based on the criteria in the document, "Biosafety in Microbiological and Biomedical Laboratories", 5th Edition, published by the Centers for Disease Control (CDC).

This document may be downloaded at

<http://www.cdc.gov/biosafety/publications/bmb15/index.htm>

Institutional Guidelines: Safety requirements for use and handling of lentiviruses may vary at individual institutions.

We recommend consulting your institution's health and safety guidelines and/or officers prior to implementing the use of these reagents in your experiments.

A detailed discussion of lentiviral vectors is provided in Pauwels, K. et al (2009). State-of-the-art lentiviral vectors for research use: Risk assessment and biosafety recommendations. *Curr. Gene Ther.* 9: 459-474.

the envelope is pseudotyped with the VSV-G gene from Vesicular Stomatitis Virus place of the HIV-1 envelope (Burns et al., 1993; Emi et al., 1991; Yee et al., 1994). Although the virus tests negative for mycoplasma, bacteria and fungi, no test procedure can guarantee the absence of known and unknown infectious agents. Consequently, all products should always be considered potentially biohazardous and appropriate precautions should be taken. Use good laboratory practice and aseptic technique at all times.

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