IncuCyte® NeuroLight Lentivirus Reagents for Labeling Live Neuronal Cells

Product Information

Presentation, storage and stability

IncuCyte® NeuroLight Lentivirus Reagents are supplied as kits containing two vials, each vial containing 0.45 mL of 3rd generation HIV-based, VSV-G pseudotyped lentiviral particles suspended in DMEM, providing sufficient quantity for 1 x 96-well plate.

Product Name	Ex Max.	Em Max.	Cat. No.	Storage Conditions	Stability
Compatible with IncuCyte Live-Cell Analysis Systems configured with a red/green optical module					
IncuCyte NeuroLight Red Lentivirus Reagent	588 nm	633 nm	4807	-80° C	6 months from date of receipt
Compatible with IncuCyte Live-Cell Analysis Systems configured with an orange/NIR optical module					
IncuCyte NeuroLight Orange Lentivirus Reagent	555 nm	584 nm	4808	-80° C	6 months from date of receipt

Safety data sheet (SDS) information can be found on our website at **www.sartorius.com**

Background

The IncuCyte NeuroLight Lentiviral reagents are third generation HIV-based, VSV-G pseudotyped lentiviral particles to enable highly-efficient, yet non-disruptive labeling of primary or iPSC-derived neurons. Once integrated into cells of interest, a neuron-specific (synapsin) promoter drives stable, long-term expression of a red or orange fluorescent protein (mKate2 or TagRFP, respectively) in neuronal cell bodies and neurites. This enables the kinetic quantification of neurite length and branching over a period of several weeks even in the presence of astrocytes and other non-neuronal cell types such as microglia.

The NeuroLight Red and Orange Lentivirus reagents have been validated for use with the IncuCyte® Live-Cell Analysis Systems for measurements of neurite outgrowth, maturation, and the disruption of neurite networks.

Recommended use

This product is designed for use in a neuronal co-culture assay format. Performance in a mono-culture format has not been validated. We recommend thawing the IncuCyte NeuroLight Lentivirus Reagents on ice immediately prior to use. The lentivirus reagents can be prepared in full media and added directly to plated cells, per the protocol below.

Example data

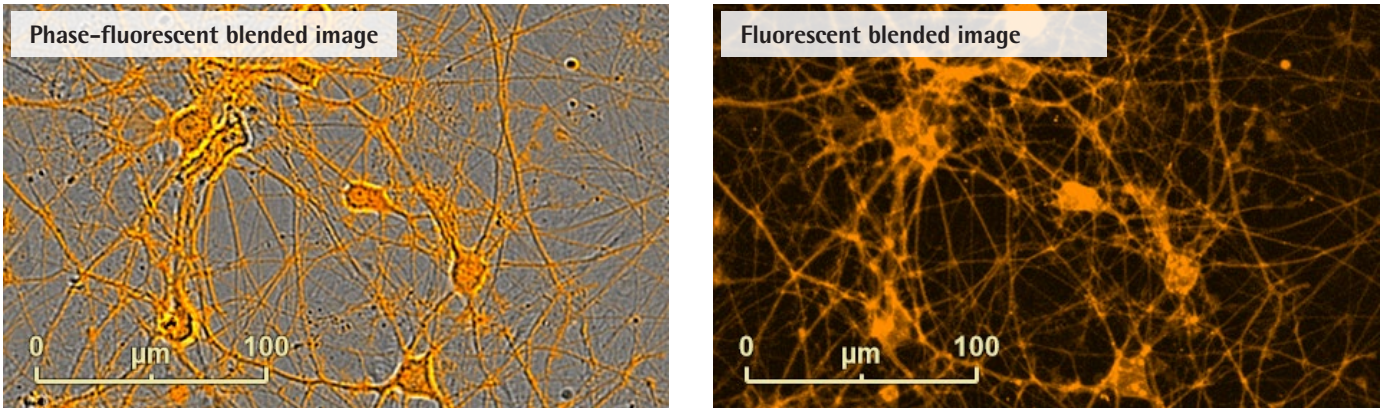
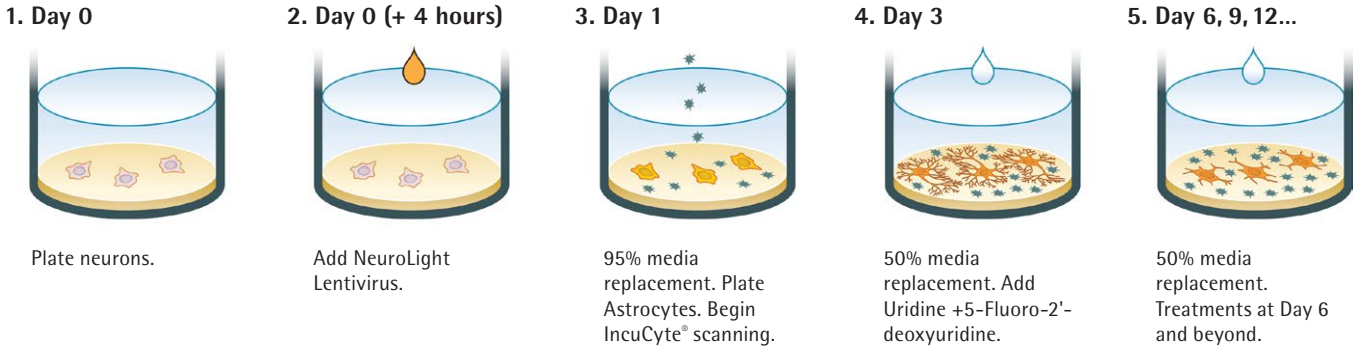


Figure 1. Representative images of rCortical neurons infected with IncuCyte NeuroLight Orange Lentivirus in a co-culture with rAstrocytes. Note the neuronal specific labeling of the orange (TagRFP) fluorescent protein and healthy cell morphology.

Quick guide



Protocols and Procedures

Materials required

Software:

- IncuCyte® NeuroTrack Software Module (Cat. No. 9600-0010)

Reagents:

- IncuCyte® NeuroLight Lentivirus Reagent
- 5-Fluoro-2'-deoxyuridine—Sigma Aldrich (Cat. No. F0503)
- Uridine—Sigma Aldrich (Cat. No. U3003)
- Surface coating materials for 96-well plate
- Neuronal cell culture medium
- Neuronal cells
- Astrocytes

NOTES:

- This product is designed for use in a neuronal co-culture assay format. Performance in a mono-culture format has not been validated.
- Use rigorous aseptic technique at all times, opening the culture plate and medium bottles within a tissue culture hood only.

Optimization protocol

We recommend optimizing NeuroLight Lentivirus volume per well for each new lot of virus or each uncharacterized cell type tested per our guidelines below. The optimization outline below will generate a 3-fold serial dilution of virus from 30–0.04 µL/well when added to a 96-well plate containing neurons seeded in a 100 µL volume.

1. Plate the desired density of neurons, 100 µL per well in a 96-well plate pre-coated with appropriate matrix. The seeding density will need to be optimized for each neuronal cell type used; however, we have found that 15,000 viable cells per well (150,000 cells/mL seeding stock) for primary neurons and iPSC-derived neurons is a reasonable starting point.

   NOTE: For immortalized cell lines, passage number can have a significant effect on lentiviral transduction efficiency. Low passage cells should be used in all experiments.

2. Let the covered plate sit at ambient temperature in the tissue culture hood for 30 minutes and then place inside the incubator.

   CRITICAL: This step ensures the uniform distribution of cells in each well.

3. Incubate the 96-well plate containing neurons at 37° C for 4 hours prior to infection.
4. 2-3 hours after plating neurons, thaw one vial of lentivirus on wet ice (approx. 1-2 hours).
5. In a sterile 96-well culture plate, create a serial dilution of NeuroLight Lentivirus using the provided plate map (Figure 2).
   a. In a sterile 2 mL vial, dilute 360 µL of NeuroLight Lentivirus in 840 µL of neuronal culture media (NCM) for a final volume of 1200 µL.
   b. Add 180 µL of diluted NeuroLight Lentivirus to wells A1-A6.
   c. Add 120 µL of NCM to wells B1-B6 and continue down to wells H1-H6.
   d. Using a multi-channel pipette, perform a 1:3 serial dilution by transferring 60 µL of lentivirus from wells A1-A6 to wells B1-B6. Pipette up and down a few times to mix reagent within each well.
   e. Continue the 1:3 dilution down the 96-well plate, stopping at row G. Row H is a no virus control, containing media only.
   NOTE: The leftover lentivirus should be used right away or be disposed, since repeated freeze-thaw cycles may decrease virus titer.
6. 4 hours post plating, remove plated neurons from incubator.
7. Gently add 100 µL per well of diluted virus from step 5, to the 96-well plate containing neurons with a final volume of 200 µL per well.
8. Return the plate to 37° C immediately and incubate the plate for 16-24 hours.
9. Before plating astrocytes, gently remove 190 µL transduction media and add 140 µL/well of appropriate neuronal medium.
10. Initiate co-culture by plating 50 µL of astrocytes on top of the infected neurons. We recommend seeding astrocytes at 15,000 viable cells per well (300,000 cells/mL seeding stock), whether astrocyte suspension is prepared from fresh stocks or cryopreserved cells.
11. Place plate into the IncuCyte® Live-Cell Analysis System and schedule to acquire phase and fluorescent images every 6 hours.
12. Approximately 48 hours post-plating astrocytes, remove 100 µL of media from each well and replace with 100 µL fresh media containing 2x concentrations of 5-Fluoro-2'-deoxyuridine and uridine to a final assay concentration of 8 µg/mL and 28 µg/mL, respectively, in order to arrest astrocyte proliferation.
13. Monitor the cultures over the next 5-12 days, performing a 50% media change every third day.
14. Analyze the neurite outgrowth with the IncuCyte® NeuroTrack Software Module. We recommend using the neurite length measurement to ensure the best determination of virus volume used for infection. The lowest virus volume that results in the highest neurite outgrowth measurement should be selected for subsequent neurite outgrowth experiments.
15. Once the optimal virus volume has been determined, calculate the virus volume required for making a 11 mL virus transduction solution sufficient to infect an entire 96-well plate:

    (volume of virus / well) x 110 wells = total amount of virus required for assay

Example data for virus optimization:

A.

All	1	2	3	4	5	6
A	Lentivirus 30 µL/well Neurons 15K/well					
B	Lentivirus 10 µL/well Neurons 15K/well					
C	Lentivirus 3.33 µL/well Neurons 15K/well					
D	Lentivirus 1.11 µL/well Neurons 15K/well					
E	Lentivirus 0.37 µL/well Neurons 15K/well					
F	Lentivirus 0.12 µL/well Neurons 15K/well					
G	Lentivirus 0.12 µL/well Neurons 15K/well					
H	No virus control Neurons 15K/well					

B.

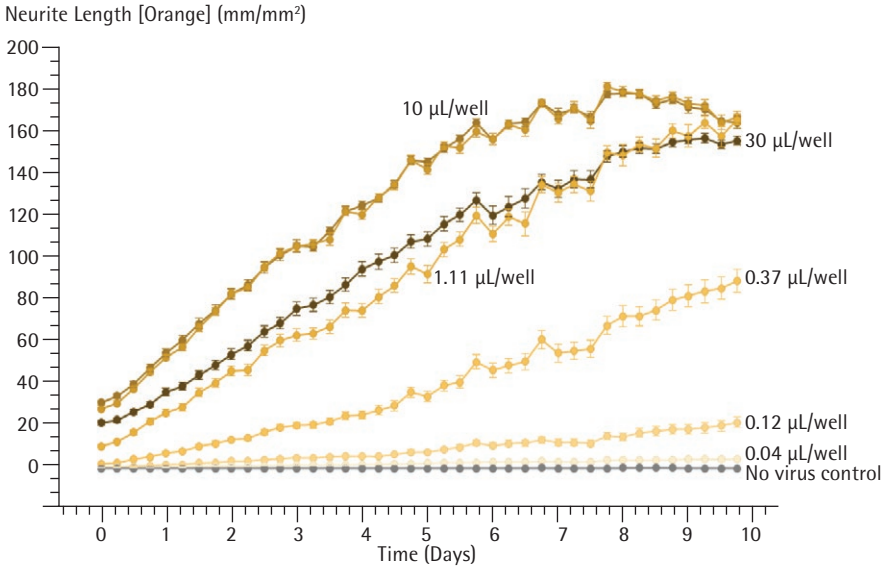


Figure 2: Schematic of NeuroLight assay optimization, highlighting recommended cell seeding and NeuroLight virus dilutions (A). Kinetic graph of neurite length showing maximum neurite length using 10 or 3.33 µL/well of NeuroLight Lentivirus (B).

Neurite co-culture protocol (post viral optimization)

1. Plate neurons at optimized density (e.g., 15,000 cells per well), 100 µL per well (150,000 cells/mL seeding stock), in a 96-well plate pre-coated with appropriate matrix. Incubate at ambient temperature for 30 minutes.
2. Place in incubator and allow 4 hours for cells to adhere.
3. 2-3 hours after plating neurons, thaw the remaining vial of lentivirus on wet ice (approx. 1-2 hours).
4. Add appropriately diluted NeuroLight Red or Orange Lentivirus to achieve optimized virus concentration. The final well volume should be 200 µL per well.
5. Incubate the 96-well plate at 37° C for 16-24 hours.
6. Before plating astrocytes, remove 190 µL transduction media and add 140 µL/well of appropriate neuronal medium.
7. Initiate co-culture by plating 50 µL of astrocytes on top of the infected neurons. We recommend seeding astrocytes at 15,000 viable cells per well (300,000 cells/mL seeding stock), whether astrocyte suspension is prepared from fresh stocks or cryopreserved cells.
8. Place plate into the IncuCyte® Live-Cell Analysis System and schedule to acquire phase and fluorescent images every 6 hours using the 20X objective. (See IncuCyte User Manual for detailed instructions on setting up an imaging schedule.)
9. Approximately 48 hours post-plating astrocytes, remove 100 µL of media from each well and replace with 100 µL fresh media containing 2x concentrations of 5-Fluoro-2'-deoxyuridine and uridine to a final assay concentration of 8 µg/mL and 28 µg/mL, respectively, in order to arrest astrocyte proliferation.
10. Monitor the cultures over the next 5-12 days, performing a 50% media change every third day.

NOTE: Transduction efficiencies of 60-70% are typical. In some cases, it may be preferred to use a lower concentration of lentivirus in order to track neurite dynamics in a high density culture.

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 (ΔU3) resulting in "self-inactivation" (SIN) of the lentivirus after transduction and genomic integration in 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.
- The virus is pseudotyped with VSV-G from Vesicular Stomatitis Virus in place 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 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 safe lentivirus handling, we recommend referring to local documentation based on geography. The Essen BioScience 3rd generation HIV based lentiviruses 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/bmbl5/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.

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Licenses and Warranty

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