



# IncuCyte® NeuroPrime Kits for Measuring Neurite Outgrowth

## Product Information

### Presentation, storage and stability

The IncuCyte® NeuroPrime Kits contain sufficient quantity of reagent for the detection of neurite dynamics in 1 x 96-well plate.

Product Name	Amount	Cat. No.	Storage Conditions	Stability
IncuCyte® NeuroPrime Red Kit		4585		6 months from date of receipt
• IncuCyte® rCortical Neurons (2 x 10 <sup>6</sup> cells/vial)	1 vial	4753	LN	
• IncuCyte® rAstrocytes (2 x 10 <sup>6</sup> cells/vial)	1 vial	4586	LN	
• IncuCyte® NeuroLight Red Lentivirus (0.45 mL/vial)	1 vial	4584	-80° C	

NeuroLight Red Lentivirus Spectral Properties:  $\lambda_{abs}/\lambda_{em} = 588/633$  nm  
Compatible with IncuCyte® Live-Cell Analysis Systems configured with a red/green optical module.

IncuCyte® NeuroPrime Orange Kit		4760		6 months from date of receipt
• IncuCyte® rCortical Neurons (2 x 10 <sup>6</sup> cells/vial)	1 vial	4753	LN	
• IncuCyte® rAstrocytes (2 x 10 <sup>6</sup> cells/vial)	1 vial	4586	LN	
• IncuCyte® NeuroLight Orange Lentivirus (0.45 mL/vial)	1 vial	4758	-80° C	

NeuroLight Orange Lentivirus Spectral Properties:  $\lambda_{abs}/\lambda_{em} = 555/584$  nm  
Compatible with IncuCyte® Live-Cell Analysis Systems configured with an orange/NIR optical module.

Safety data sheet (SDS) information can be found on our website at [www.sartorius.com](http://www.sartorius.com)

### Background and recommended use

The IncuCyte® NeuroPrime Kits contain cryopreserved vials of rat cortical neurons and astrocytes isolated from embryonic stage (E18) Sprague-Dawley rats as well as sufficient NeuroLight Red or Orange Lentivirus to perform one 96-well experiment. The lentivirus encodes a red (mKate2) or orange (TagRFP) fluorescent protein regulated by a synapsin promoter that selectively drives expression in neurons and minimizes expression in non-neuronal

cell types. After 6 days in co-culture, the neurons form extensive neurite networks enabling the neurotoxic or neuroprotective effects of treatments to be assessed. Dynamic changes in neuronal network length and branching are measured using IncuCyte® Live-Cell Analysis Systems.

## Example data

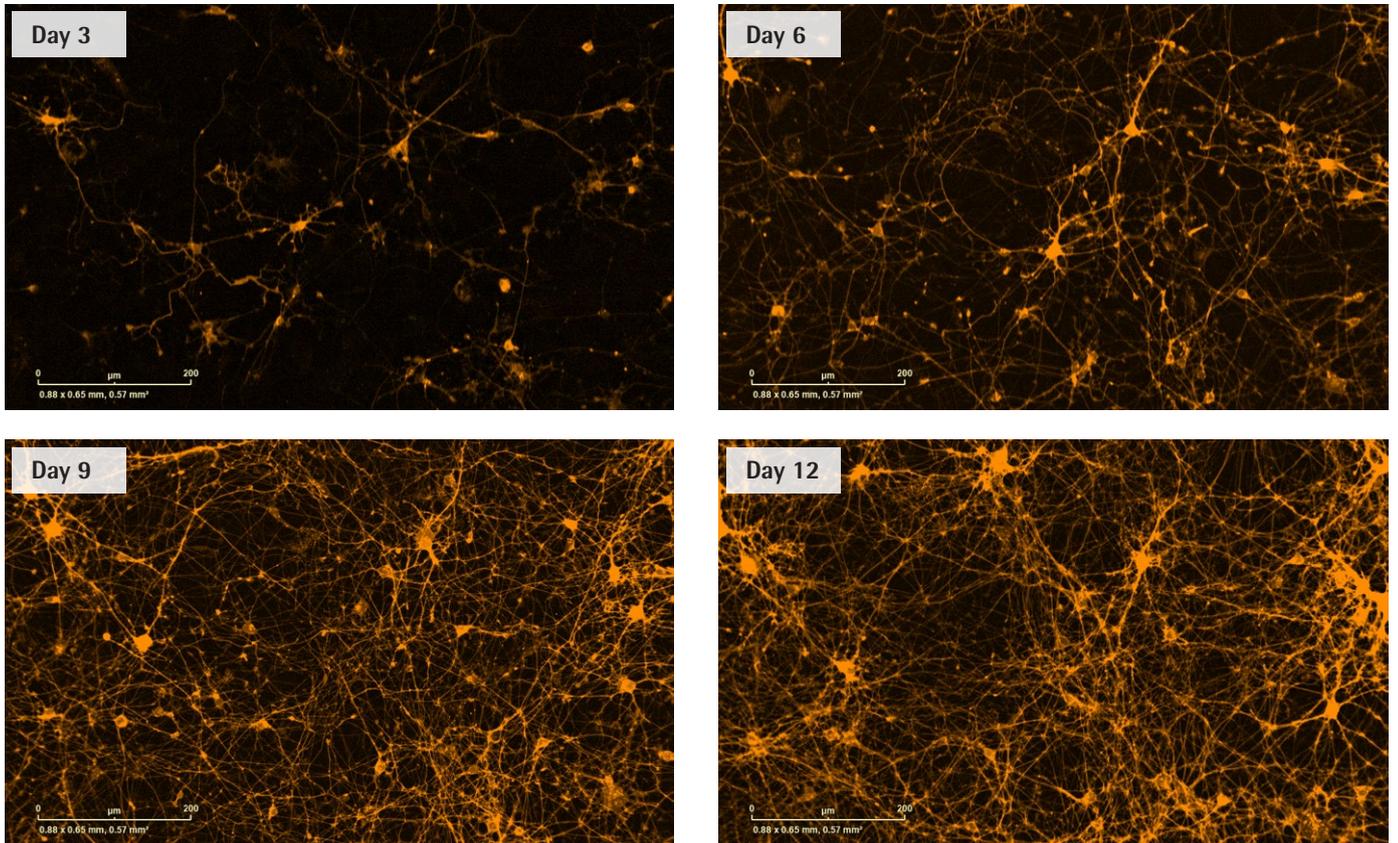


Figure 1. Representative images of rCortical Neurons transduced with NeuroLight Orange Lentivirus in co-culture with rAstrocytes, showing neuron-specific labeling over time.

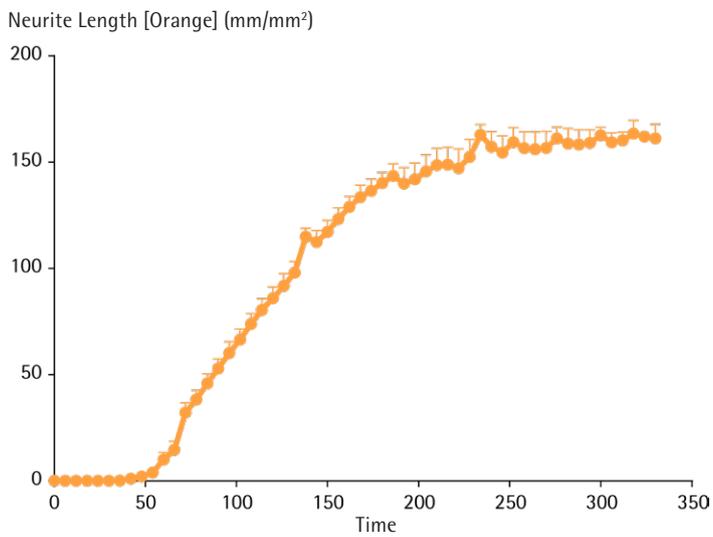


Figure 2. Time course analysis of neurite outgrowth of rCortical cells labeled with the IncuCyte® NeuroLight Orange Lentivirus.

## Quick guide

### 1. Day 0

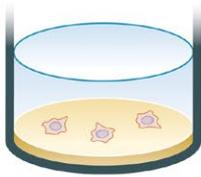
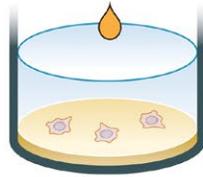


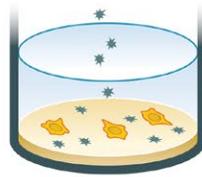
Plate rCortical neurons.

### 2. Day 0 (+ 4 hours)



Add NeuroLight Lentivirus.

### 3. Day 1



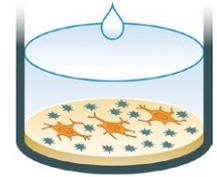
95% media replacement. Plate rAstrocytes. Begin IncuCyte® scanning.

### 4. Day 3



50% media replacement. Add Uridine +5-Fluoro-2'-deoxyuridine.

### 5. Day 6, 9, 12...



50% media replacement. Treatments at Day 6 and beyond.

## Protocols and Procedures

### Materials

- IncuCyte® NeuroPrime Red or Orange Kit

### Materials required but not provided:

- Poly-D-Lysine—Millipore (Cat. No. A-003-E)
- Sterile WFI water—Corning CellGro Mediatech (Cat. No. 25-055-CM)
- Neurobasal Media—Life Technologies (Cat. No. 21103049)
- B-27 Serum Free Supplement—Life Technologies (Cat. No. 17504044)
- GlutaMAX-I Supplement—Life Technologies (Cat. No. 35050061)
- DMEM—Life Technologies (Cat. No. 11965 or 41965)
- Fetal Bovine Serum—Sigma Aldrich (Cat. No. F2442) or Thermo Scientific (Cat. No. SH3007103)
- 5-Fluoro-2'-deoxyuridine—Sigma Aldrich (Cat. No. F0503)
- Uridine—Sigma Aldrich (Cat. No. U3003)

### Software:

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

### Solutions to prepare in advance

#### Day -1

Poly-D-Lysine

- 100 µg/mL in 12 mL of WFI quality water

#### Day 0

Neuronal Culture Media (NCM)—for 50 mL

- 48.5 mL Neurobasal Media
- 0.5 mL GlutaMAX-I
- 1 mL B-27 Supplement

#### Day 1

Neuronal Culture Media (NCM)—for 50 mL

- 48.5 mL Neurobasal Media
- 0.5 mL GlutaMAX-I
- 1 mL B-27 Supplement

Astrocyte Culture Media—for 50 mL

- 42.5 mL DMEM
- 7.5 mL FBS

#### Day 3

2x 5-Fluoro-2'-deoxyuridine and Uridine (FdU/U)

- Dissolve 8 mg of FdU and 28 mg of U in 100 mL Neurobasal media to make 10x FdU/U stock solution.
- Dilute to 2x FdU/U in total volume of 12 mL in a 15 mL conical tube.
  - Add 2.4 mL 10x FdU/U
  - Add 9.6 mL NCM
- 10x FdU/U stock solution can be aliquoted and stored at -20° C for future use, at which point it can be thawed on ice.

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

#### Day -1: Coat 96-well plate with Poly-D-Lysine

1. Coat one 96-well plate with Poly-D-Lysine. Prepare a 100 µg/mL stock of Poly-D-Lysine in sterile WFI quality water and add 100 µL to each well. Replace lid and incubate for 16–20 hours at ambient temperature in the tissue culture hood.

#### Day 0: Thaw and plate rCortical Neurons

1. Aspirate and discard the Poly-D-Lysine and rinse the plate twice with 150 µL/well of sterile WFI water. If excess Poly-D-Lysine is not washed away it can impair neurite outgrowth.
2. Leave the plate to dry for at least one hour with lid removed in the tissue culture hood.
3. Prepare the Neuronal Culture Media (NCM). For 50 mL of complete NCM, add 1 mL of B-27 Serum Free supplement, 0.5 mL GlutaMAX-I to 48.5 mL of Neurobasal media in a 50 mL conical tube.

**CRITICAL: Warm NCM to 37° C prior to thawing neurons.**

4. Remove the vial of rCortical Neurons from liquid nitrogen storage and thaw in a 37° C water bath until only a tiny ice crystal remains (1 to 2 minutes).

**CRITICAL: Do not agitate the vial during this step.**

5. Wipe outside of vial with 70% ethanol.
6. In tissue culture hood, use a P1000 pipette to pre-wet a tip with 1 mL NCM.
7. Use the pre-wetted tip to transfer the 1 mL volume of thawed neuronal cells to a 50 mL conical tube.
8. Rinse the cryo-vial with 1 mL NCM and slowly transfer the rinse media in a drop-wise fashion to the 50 mL conical tube containing neurons, while gently swirling the 50 mL conical tube.
9. **CRITICAL: Rapid addition of the media at this point can result in osmotic shock and cell death. The 1 mL addition should take about 30 seconds.**
10. In drop-wise fashion, slowly add a further 2 mL pre-warmed NCM to the 50 mL conical tube. The 2 mL addition should take about one minute.
11. Perform a cell count (e.g. Trypan Blue staining with hemocytometer) and dilute neurons to 150,000 cells/mL or desired density in pre-warmed NCM. The cell suspension can be transferred to a sterile trough in the tissue culture hood at this point in order to facilitate pipetting of cells in the next step.
12. Using a multichannel handheld pipette, dispense 100 µL of neuronal cell suspension into each well of the Poly-D-Lysine coated 96-well plate (15,000 neurons/well).
13. **CRITICAL: To ensure proper mixing and uniform seeding of the neurons, mix the cell suspension by gently pipetting up and down 1–2 times between seeding each row of the plate. Rocking the trough is also recommended to ensure equal cell distribution.**
14. Let the covered plate sit at ambient temperature in the tissue culture hood for 30 minutes and then place inside the incubator.
15. **CRITICAL: This step ensures the uniform distribution of cells in each well.**
16. Allow cells to settle on the plate for 2–4 hours before proceeding.

#### Infect neurons with NeuroLight Lentivirus

1. Allow the NeuroLight Lentivirus to thaw on ice (approximately 1 hour).
2. Prepare a 2X lentivirus working stock by combining 0.360 mL of NeuroLight lentivirus with 10.64 mL of NCM for a total volume of 11 mL.
3. Using a multichannel pipette, gently add 100 µL of the 2X lentivirus working stock to each well. The final well volume should be 200 µL per well.

**NOTE:** Infection control for the IncuCyte NeuroLight Lentivirus is the ability to efficiently infect IncuCyte rCortical Neurons to express mKate2 or TagRFP, driven by a synapsin promoter and selectively expressed in neurons, such that a volume of 3.2 µL/15,000 neurons results in a neurite length of > 50 mm/mm<sup>2</sup> in a neurite outgrowth assay (rCortical Neurons/rAstrocytes co-culture experiment).

**CRITICAL: Do not pipette up and down after adding the virus solution as this may result in damage to the plated neurons.**

4. Incubate plate in a tissue culture incubator (37° C, 5% CO<sub>2</sub>) for 16–24 hours.

#### Day 1: Plate rAstrocytes

1. Pre-warm NCM to 37° C.
  2. Carefully remove 190 µL of medium per well using a multichannel pipette, and replace immediately with 140 µL of fresh, pre-warmed NCM. Volume should now be 150 µL per well.
  3. Prepare 50 mL Astrocyte Culture Media (85% DMEM + 15% FBS; ACM) by adding 7.5 mL FBS to 42.5 mL DMEM and warm to 37° C.
  4. Remove the vial of rAstrocytes from liquid nitrogen storage and thaw in a 37° C water bath until only a tiny ice crystal remains (1 to 2 minutes).
  5. Wipe vial with 70% ethanol.
  6. In tissue culture hood, use a P1000 pipette to pre-wet a tip with 1 mL ACM.
  7. Use the pre-wetted tip to transfer the 1 mL volume of thawed astrocytes to a 50 mL conical tube.
  8. Rinse the cryo-vial with 1 mL ACM and slowly transfer the rinse media in a drop-wise fashion to the 50 mL conical tube containing astrocytes while gently swirling.
- CRITICAL: Rapid addition of the media to the cell suspension at this point can result in osmotic shock and cell death.**
9. Slowly add 2 mL pre-warmed ACM to the 50 mL conical tube in a drop-wise fashion. The 2 mL addition should be performed slowly, taking at least 1 minute.
  10. Centrifuge the astrocytes at 250 x g for 5 min. Carefully aspirate and discard the supernatant. Resuspend the cell pellet in 5 mL of ACM using a P-1000 handheld pipette by gently aspirating and dispensing 10–15 times to ensure a single suspension. Perform a cell count (e.g. Trypan Blue staining with hemocytometer) and dilute cells to 300,000 cells/mL in pre-warmed ACM.

11. Using a multichannel handheld pipette, plate 50  $\mu$ L of astrocyte cell suspension into each well of the 96-well plate containing the cultured neurons (i.e. 15,000 astrocytes/well).

**CRITICAL: To ensure proper mixing and uniform seeding of the astrocytes, mix the cell suspension by gently pipetting up and down 1–2 times between seeding each row of the plate. Rocking the trough is also recommended to ensure equal cell distribution.**

12. Place plate into the appropriate IncuCyte<sup>®</sup> instrument and schedule to image every 6 hours using the 20X objective. (See IncuCyte User Manual for detailed instructions on setting up an imaging schedule.)

#### Day 3: Treat cells with 5-Fluoro-2'-deoxyuridine and Uridine

**CRITICAL: Addition of 5-Fluoro-2'-deoxyuridine and Uridine (FdU/U) prevents over-proliferation of non-neuronal cell types.**

1. Remove 100  $\mu$ L of media from each well using a multichannel pipette and replace with 100  $\mu$ L fresh NCM containing a 2X concentration of FdU/U to a final assay concentration of 8  $\mu$ g/mL and 28  $\mu$ g/mL, respectively.
2. Return plate to incubator.

#### Days 6, 9, 12 and beyond: Solutions Required

1. Neuronal Culture Media—for 50 mL
  - a. 48.5 mL Neurobasal Media
  - a. 0.5 mL GlutaMAX I
  - a. 1 mL B-27 Supplement

#### Feeding cultures

1. Feed cultures with fresh NCM by performing a 50% media change. To do this, remove 100  $\mu$ L per well and replace with 100  $\mu$ L of fresh media.

**CRITICAL: Only a single FdU/U treatment is required (Day 3, step 1). Addition of fresh FdU/U is not recommended on following days.**
2. Cultures can be stopped at Day 11 or continued for desired length, with 50% media changes occurring every third day.

#### Analysis guidelines

Run a NeuroTrack analysis job for the appropriate fluorescent channel to quantify Neurite Length.

#### Suggested analysis parameters for NeuroTrack:

Segmentation Mode: Texture

Min Cell Width: 15.0

Neurite Sensitivity: 0.5

Neurite Width: 1

#### 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 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.

#### Biohazard note

The rCortical Neurons and rAstrocytes contain cells of rodent origin. Although the cells test 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.

## FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE.

### Licenses and Warranty

Essen BioScience products, IncuCyte® NeuroLight Orange Lentivirus Reagent and IncuCyte® NeuroLight Red Lentivirus Reagent, contain proprietary nucleic acid(s) coding for proprietary fluorescent protein(s) being, including its derivatives or modifications, the subject of pending patent applications and/or patents owned by Evrogen JSC (hereinafter "Evrogen Fluorescent Proteins"). The purchase of Essen BioScience products incorporating these fluorescent proteins conveys to the buyer the non-transferable

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