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Product Information: CellPlayer™ NeuroLight™ Red Lentivirus - Synapsin Promoter

Catalog Number: 4584

Contents

1x vial of CellPlayer™ NeuroLight Red™ Lentivirus - Synapsin promoter (0.45 mL/vial)

Lot #:

Titer:

Storage and Stability

The NeuroLight Red™ lentivirus 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

NeuroLight Red is a lentiviral based live-cell neuronal labeling reagent driven by a synapsin promoter, resulting in the long term expression of red fluorescent protein (mKate2) in neuronal cell bodies and neurites. NeuroLight Red™ 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.

Virus Description

3rd generation HIV-based, VSV-G pseudotyped lentiviral particles encoding a red fluorescent protein (mKate2).

Promoter: Synapsin

Spectral Properties: Ex (max): 588 nm; Em (max): 633 nm

Third generation lentiviral-based vectors are commonly used to transfer genetic information to cells for gene therapy and/or research purposes. The Essen BioScience CellPlayer™ NeuroLight Red lentiviral-based reagent has been specially designed to efficiently transduce multiple neuronal cell types with low toxicity. The NeuroLight Red™ lentivirus encodes a red 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 red 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 ZOOM™ and NeuroLight reagent provide an integrated solution for kinetically measuring neurite dynamics *in vitro*.

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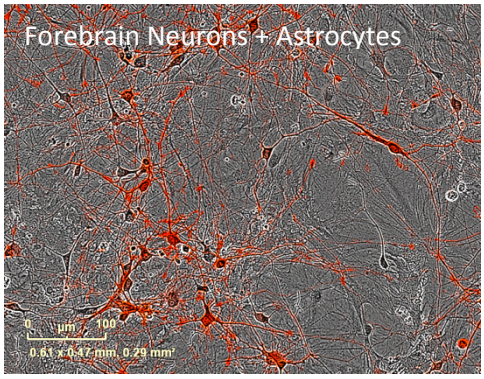
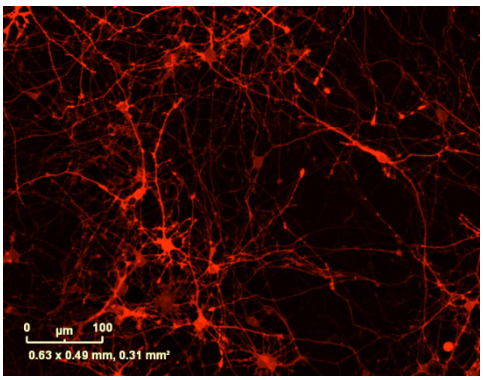
Example data

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 ZOOM images of the CellPlayer™ NeuroPrime™ Rat Forebrain Neurons in co-culture with rat astrocytes. **Left:** Phase-contrast/fluorescent blended image showing forebrain neurons infected with NeuroLight Red; **Right:** Fluorescent image of Rat Forebrain neurons expressing NeuroLight Red, same field of view as image on left. Scale bar: 100µm.

CellPlayer™ NeuroLight™ Red Lentivirus Protocol Overview - for neuronal and astrocyte co-cultures

Day 0	Day 0 (+4 hours)	Day 1	Day 3	Day 6, 9, 12, …

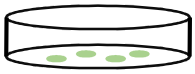
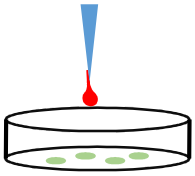
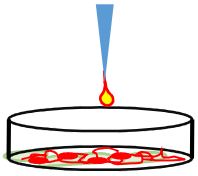
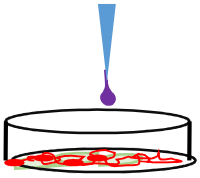

				

Plate neurons	Add NeuroLight™ Red Lentivirus	95% media Replacement Plate astrocytes Begin IncuCyte scanning	50% media replacement Add Uridine + 5-FDUridine	50% media replacement Treatments @ day 6 and beyond

Materials Required But Not Provided

Equipment

- 96-well plate, flat-bottom – Corning (Cat. No. 3595) or TPP (Cat. No. 92096)
- Class 2 Tissue culture hood
- Multichannel hand-held pipette (Sufficient for up to 200 µL per well)
- P-1000 hand held pipette
- Sterile containers for preparing test compound dilutions
- Tissue culture incubator (37°C, 5% CO_2, humidified atmosphere)
- IncuCyte ZOOM with CellPlayer™ NeuroTrack™ Software Module (Cat.# 9600-0010)
- Water bath set to 37°C

Reagents

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

! CRITICAL Use rigorous aseptic technique at all times. Only open the culture plate and medium bottles within a in 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 Red reagent to achieve desired MOI (see Appendix for more information on determining MOI). The final well volume should be 200 µL per well.
3. Incubate the 96-well plate at 37° C for 16-24 hours.
4. Remove 190 µL transduction media and add 140 µL/well of appropriate neuronal medium.
5. Initiate co-culture by plating 50 µL 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 ZOOM™ imaging system, to initiate assay.
7. 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.
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 MOI in order to track neurite dynamics in a high density culture.

Related Products

Catalog # 4585	CellPlayer™ NeuroPrime™ Cell Kit
Catalog # 4586	CellPlayer™ NeuroPrime™ rAstrocytes
Catalog # 4587	CellPlayer™ NeuroPrime™ rForebrain Neurons
Catalog # 9600-0010	CellPlayer™ NeuroTrack™ Software Module

For additional information on this and other products, please contact Essen BioScience at: sales@essenbio.com.

Appendix

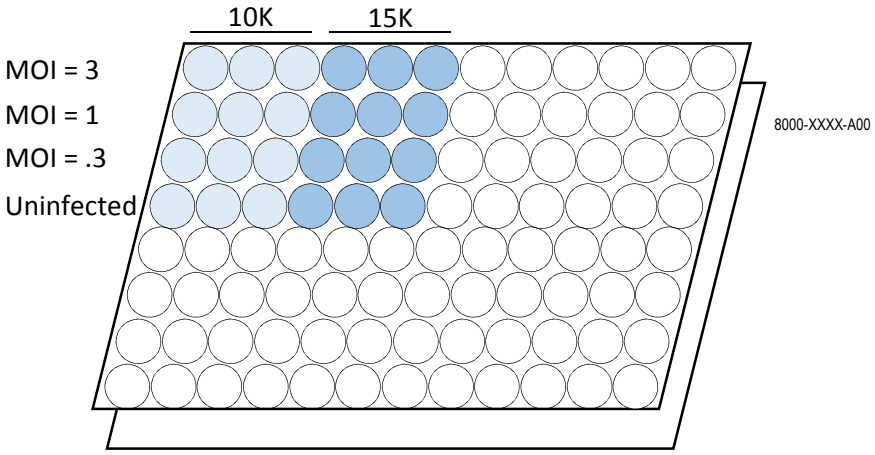
Optimizing Multiplicity of Infection (MOI)

Method to empirically determine the optimal MOI for your primary or iPSC-derived neurons.

Protocol: Lentiviral Infection of primary or iPSC-derived neurons

1. Plate at least two densities of cells in the appropriate plate (10 and 15K for primary neurons or 15 and 20 K for iPSC neurons). We recommend optimizing in a 96-well plate.

   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. 2-3 hours after seeding neurons, thaw lentivirus on ice (approx. 1-2 hours).

3. Dilute lentivirus to the appropriate MOI in the appropriate media sufficient for delivering 100 µL/well.

   Example: For neurons seeded at 15,000 cells/well (# of neurons), dilute virus in 11 mL of appropriate media to MOI=1. This will be sufficient for a single 96-well plate at 100 µL/well (volume delivered per well).

   a. To calculate the volume of virus required for sufficient volume to infect a single plate (total volume of media), use the following equation:

$$volume\ of\ virus = \left[\frac{total\ volume\ of\ media}{\left[\frac{viral\ titer}{\#\ of\ neurons}\right] * volume\ delivered\ per\ well}\right] * MOI$$

      i. Viral titer information can be found on the NeuroLight Red™ Product Data Sheet

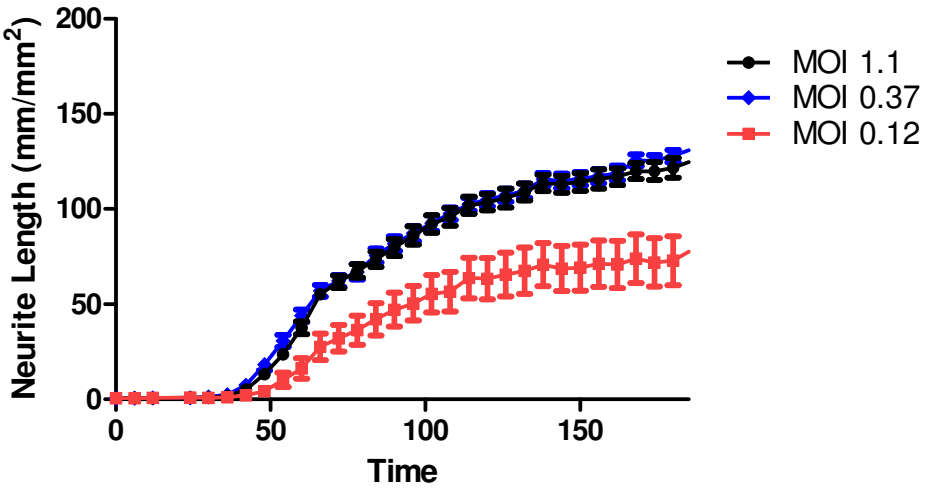
b. Recommended volumes and cell densities for 96-well plates:

Plate Type	# of Neurons/well	Neuron Seeding Volume	Lentivirus volume delivered per well	Final Volume
96-well plate	10,000 - 20,000	100 µL	100 µL	200 µL

4. 4 hours post plating, remove plated neurons from incubator.

5. Add appropriate volume of virus solution per well (at appropriate MOI) and return to incubator immediately.

6. Once the optimal MOI has been determined, see applicable section of protocol for setting up either primary or iPSC-derived neuron/astrocyte co-cultures.



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 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 psueudotyped 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 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/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.

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.

Biohazard Note

The NeuroLight Red™ 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 (ΔU3) 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, the envelope is psueudotyped 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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