

IncuCyte® NeuroBurst Orange Lentivirus—Synapsin Promoter

Catalog number: 4736

Contents

- 1 vial of IncuCyte® NeuroBurst Orange Lentivirus - Synapsin promoter (2 mL/vial)

Storage and stability

The NeuroBurst 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 NeuroBurst Orange Reagent is a lentiviral based live-cell neuronal labeling reagent driven by a synapsin promoter, resulting in the long term expression of a genetically encoded orange fluorescent calcium indicator (mRuby-based) in neuronal cells. NeuroBurst 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 neuronal activity and functional connectivity in the presence or absence of astrocytes.

The NeuroBurst 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 Neuroburst Orange Reagent is not compatible with IncuCyte instruments configured with a Red/Green Optical Module, e.g. Cat. No. 4647. The NeuroBurst Orange Reagent is intended for use with the IncuCyte® S3 Neuronal Activity Software Module (Cat. No. 9600-0032).

Virus description

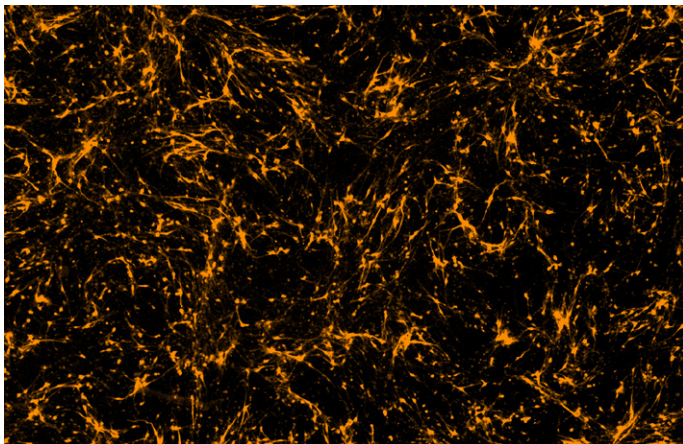
Third generation HIV-based, VSV-G pseudotyped lentiviral particles encoding a genetically encoded orange fluorescent calcium indicator.

- Promoter: Synapsin
- Spectral Properties: Ex (max): 558 nm; Em (max): 605 nm

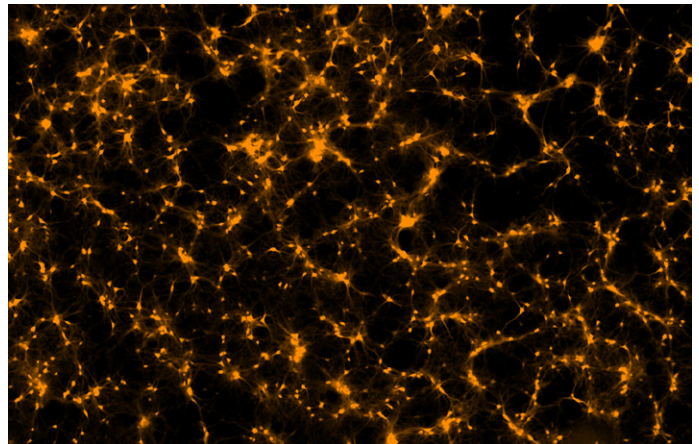
Third generation lentiviral-based vectors are commonly used to transfer genetic information to cells for gene therapy and/or research purposes. The IncuCyte NeuroBurst Orange lentiviral-based reagent has been specially designed to efficiently transduce multiple neuronal cell types with low toxicity. The NeuroBurst Orange Reagent a genetically encoded calcium indicator 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 NeuroBurst Orange Reagent provide an integrated solution for kinetically measuring neurite dynamics *in vitro*.

Example data

NOTE: This product is designed for use with the IncuCyte® S3 Live-Cell Analysis System for Neuroscience and the IncuCyte® S3 Neuronal Activity Software Module.



Fluorescent image showing iPSC derived neurons infected with NeuroBurst Orange Reagent (DIV15).

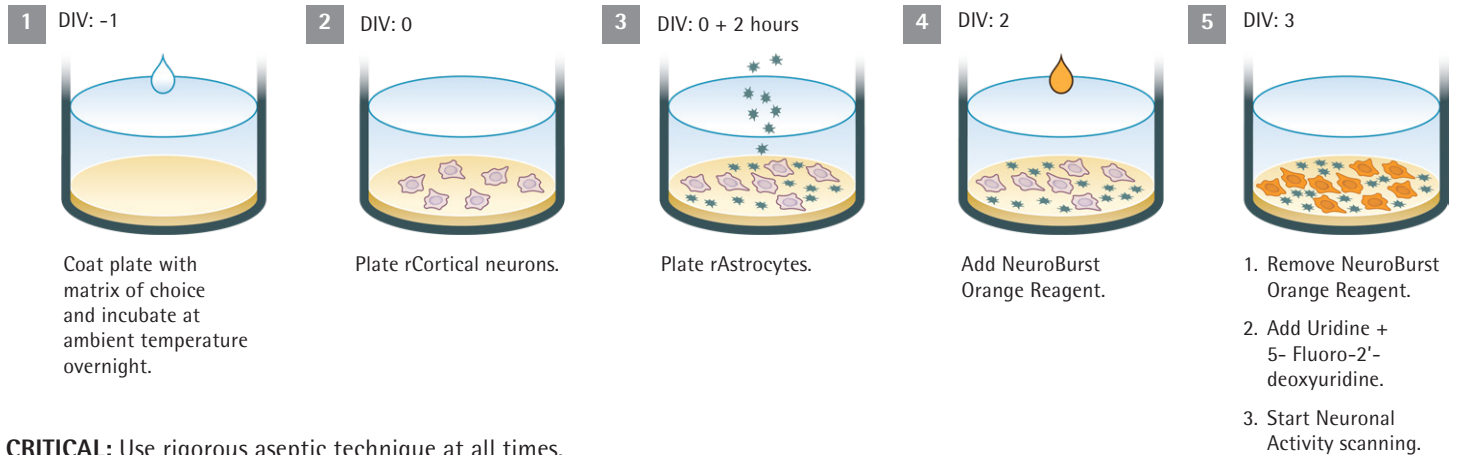


Fluorescent image showing rat cortical neurons infected with NeuroBurst Orange Reagent (DIV15).

Protocols: IncuCyte® NeuroBurst Orange Reagent

Protocol Overview: IncuCyte® NeuroBurst Orange Reagent for neuronal activity and functional connectivity measurements

Quick Guide



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

NOTE: Quality control for the IncuCyte NeuroBurst Orange Reagent is the ability to efficiently infect IncuCyte® rCortical Neurons to express the mRuby-based IncuCyte NeuroBurst Orange Lentivirus driven off of the synapsin promoter, such that a concentration of > 3.7 $\mu\text{L}/20,000$ neurons results in an active object count > 500 at day 10 in a Neuronal Activity Assay (rCortical Neurons/rAstrocyte co-culture experiment). We recommend performing a volumetric titration from 100-0.14 μL for each neuronal cell line evaluated. Evaluation of neuronal activity is to be performed on an IncuCyte S3 for Neuroscience.

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 packageable 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 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/bml5/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 NeuroBurst Orange Reagent contains 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, 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.

Licences and warranty

Essen BioScience warrants that this product performs as described on the product label and in all literature associated with the sale of said product when used in accordance with the described protocol. This limited warranty is the sole warranty. No other warranties exist that extend beyond this warranty, either expressed or implied. Essen BioScience disclaims any implied warranty of merchantability or warranty of fitness for a particular purpose. Essen BioScience disclaims any responsibility for injury or damage and shall not be liable for any proximate, incidental or consequential damages in connection with this product. If it is proven to the satisfaction of Essen BioScience that this product fails to meet performance specifications, Essen BioScience's sole obligation, at the option of Essen BioScience, is to replace the product or provide the purchaser with credit at or below the original purchase price. This limited warranty does not extend to anyone other than the purchaser. Notice of suboptimal performance must be made to Essen BioScience within 30 days of receipt of the product.

1. IncuCyte® NeuroBurst Orange is covered under US Patents #14/350,199; #8,629,256, #14/800,814, #14/800,814, #14/941,406, #14/974,483, 14/941,406, 14/974,483, 14/452,428 and foreign equivalents and licensed from Janelia Research Campus, HHMI, Janelia, Virginia, USA.
2. The products and the reagents generated from these services shall be used as tools for research purposes, and shall exclude any human or clinical use.
3. The purchase of the IncuCyte® NeuroBurst Orange Reagent conveys to the purchaser the limited, non-transferable right to use the products purchased and the reagents generated from Essen BioScience and any related material solely for Research Purposes only, not for any Commercial Purposes.