SARTORIUS

Product Guide

Incucyte® Cytolight Lentivirus Reagents for Cytoplasmic Labeling

Create Stable Cell Populations or Clones Expressing a Cytoplasmic Fluorescent Label

Product Information

Presentation, Storage and Stability
The Incucyte® Cytolight Lentivirus Reagents are supplied as 0.6 mL or 0.2 mL vials of 3rd generation HIV-based, VSV-G pseudotyped lentiviral particles suspended in DMEM. The lentivirus reagents should be stored at -80° C. When stored as described, the Incucyte® Cytolight Lentivirus will be stable for at least 3 months from the date of receipt.

Background and Intended Use
Incucyte® Cytolight Lentivirus enables efficient, non-perturbing cytoplasmic labeling of living mammalian cells. Incucyte® Cytolight Lentivirus provides homogeneous expression of a cytoplasmic GFP (green fluorescent protein) or mKate2 (red fluorescent protein) in your choice of primary, immortalized, dividing, or non-dividing cells without altering cell function and with minimal toxicity. These reagents are ideal for generating stable cell populations or clones using puromycin selection. The Incucyte® Cytolight Lentivirus has been validated for use with the Incucyte® Live-Cell Analysis System.

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

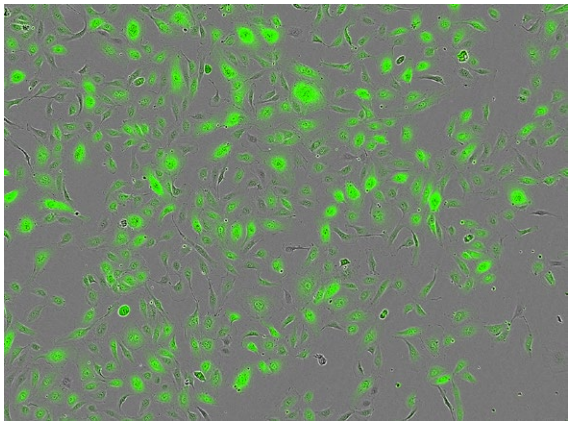
Recommended Use

We recommend that the Incucyte® Cytolight Lentivirus is thawed on ice and working aliquots are stored at -80° C. Excessive freeze | thaw cycles can impair transduction efficiency. The lentivirus reagents can be prepared in full media and added directly to plated cells for transduction. We recommend an MOI of 3 to 6, depending on the cell type being transduced, and the cationic polymer Polybrene® may be added to further enhance transduction efficiency. When used with the Incucyte® Live-Cell Analysis System, we recommend data collection every 2 hours.

For viral titer and lot information please visit our web page at **www.essenbioscience.com/lentivirus-viral-titers**

Product	Cat. No.	Promoter	Amount	Ex. Maxima	Em. Maxima
Incucyte® Cytolight Green Lentivirus (EF-1α, Puro)	4481	EF-1α	0.6 mL	483 nM	506 nM
Incucyte® Cytolight Red Lentivirus (EF-1α, Puro)	4482	EF-1α	0.6 mL	588 nM	633 nM
Incucyte® Cytolight Green Lentivirus (CMV, No selection)	4513	CMV	0.6 mL	483 nM	506 nM

Incucyte® Cytolight Green HUVEC



Incucyte® Cytolight Red A549

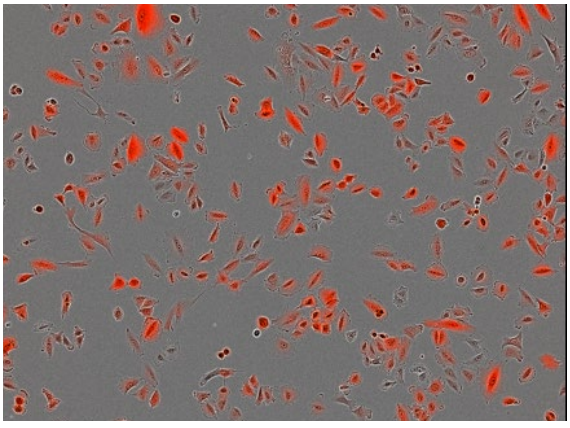
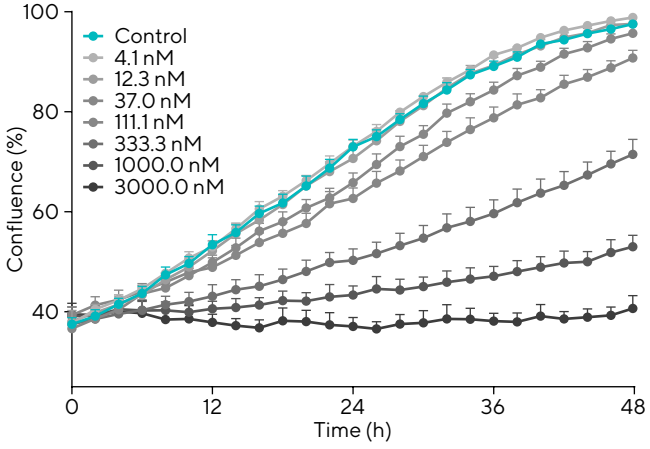


Figure 1: Representative images of primary HUVEC cells (expressing GFP) and tumor A549 cells (expressing mKate2) transduced with the Incucyte® Cytolight Lentivirus Reagents

A.



B.

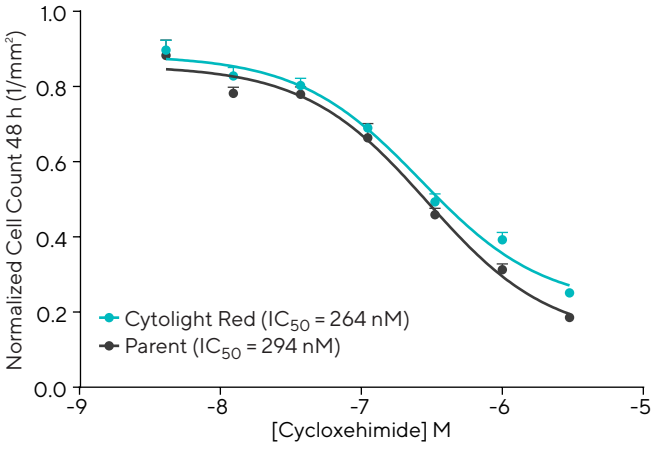
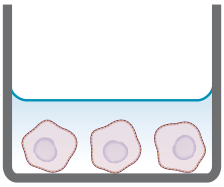


Figure 2: Concentration-dependent inhibition of proliferation by the protein biosynthesis inhibitor cycloheximide in A549 Human Lung Carcinoma Epithelial Cells labeled with the Incucyte® Cytolight Red Lentivirus. (A) Time-course of fluorescent confluence in the presence of increasing concentrations of cycloheximide. (B) After 48 h treatment, both uninfected and Incucyte® Cytolight Red Lentivirus labeled A549 cells were stained with 1 µM Vybrant® DyeCycle™ Green (ThermoFisher) and total object counts were normalized to the maximum total objects in each population. Data reveals equivalent pharmacology between labeled and uninfected cells.

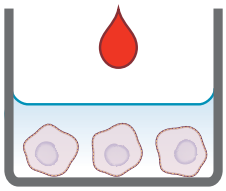
Quick Guide

1. Seed cells



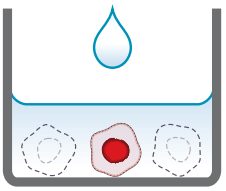
Cell Seeding
Seed cells in growth media and leave to adhere (4–24 hours). Cells should be 15–35% confluent at the time of transduction.

2. Transduce



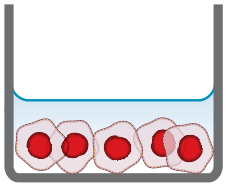
Add Incucyte® Cytolight Lentivirus
Add Incucyte® Cytolight Lentivirus (MOI 3 to 6) diluted in media ± Polybrene®. After 24 hours, replace the media with fresh growth media. Monitor expression using the Incucyte® Live-Cell Analysis System.

3. Apply selection



Generate a Stable Population or Clone
Apply antibiotic selection to derive a stable, homogenous cell population or clone that expresses a cytoplasmic fluorescent protein.

4. Live-cell fluorescent imaging



Automated Imaging and Quantitative Analysis
Capture images every 1 to 2 hours (4X, 10X or 20X) in an Incucyte® Live-Cell Analysis System. Analyze using integrated software.

Protocols and Procedures

Materials

Required Materials

- Incucyte® Cytolight Lentivirus
- Flat bottom 96-well tissue culture plate (e.g., Corning Cat. No. 3595)
- Complete cell culture media for cell line of choice

Optional

Polybrene® (Sigma H9268)
Poly-L-Ornithine (Sigma P4957)—optional, for non-adherent cell types

Suggested Infection Protocol for Immortalized Cell Lines

If you plan to use the Incucyte® Cytolight Lentivirus to generate stably expressing clones or populations, please perform the "Optimizing Antibiotic Selection" step first. Optimizing MOI and transduction conditions are less important as the selection process will eliminate non- or low-expressing cells within the population.

1. Seed cells in growth media of choice at a density such that they are 15–35% confluent at time of infection. Incubate for 24 hours or until cells have attached to the plating surface.
2. Add Incucyte® Cytolight Lentivirus at desired multiplicity of infection (MOI = TU/cell) diluted in media ± Polybrene®. An MOI of 3 and Polybrene® concentration of 8 µg/mL is recommended for most cell types.
3. Incubate at 37° C, 5% CO_2 for 24 hours.
4. After incubation, remove media and replace with fresh growth media. Return to incubator for an additional 24–48 hours, monitoring expression using an Incucyte® Live-Cell Analysis System.
5. Harvest cells and expand, freeze, or seed at desired density for subsequent experiments. For stable selection, proceed to Step 6.
6. (Optional) Remove media and replace with fresh growth media containing appropriate antibiotic selection, i.e., puromycin, at the concentration determined from the kill curve (see section below, "Optimization Protocols, Antibiotic Selection").
7. Incubate for 72–96 hours, replacing media every 48 hours.
8. Maintain stable population in a maintenance concentration of selection media (e.g., complete media containing 0.5 µg/mL puromycin).

Suggested Infection Protocol for Primary Cells and Transient Assays

If you do not plan to use the Incucyte® Cytolight Lentivirus to create stably expressing cells, we recommend optimizing MOI and Polybrene® concentration for each cell type used (see "Optimization Protocols" section below). Once these steps are complete, follow the "Suggested Infection Protocol for Immortalized Cell Lines", Steps 1 through 5.

Optimization Protocols

Antibiotic Selection (Optional)

To determine the lowest concentration of antibiotic selection required to efficiently eliminate non-transduced cells, perform a kill curve using several concentrations of puromycin for your Incucyte® Cytolight Lentivirus.

Polybrene® Concentration

The cationic polymer, Polybrene®, may be used to increase the efficiency of transduction of certain cell types. Optimal Polybrene® concentrations will vary depending on the cell type used. The following table provides recommended transduction conditions for several common cell types. Please note, Polybrene® can be toxic to certain cell types (e.g., primary neurons). The Incucyte® Cytotoxicity Assay can be used to evaluate the toxic effect of Polybrene ®on your cells.

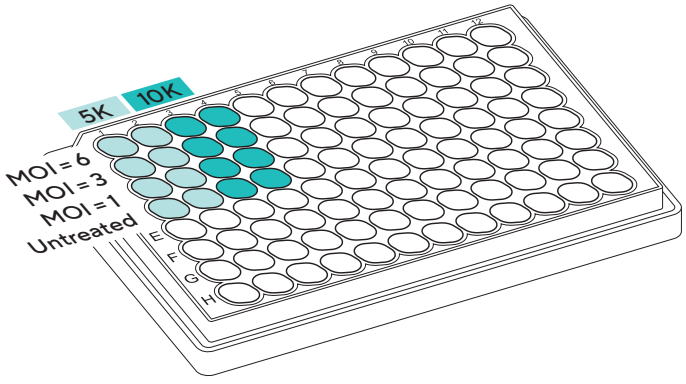
Cell line	Origin	MOI	Polybrene conc.
A549	Human lung carcinoma	3	8 µg/mL
Dermal fibroblasts	Human primary dermal fibroblast	3	5 µg/mL
ECFC	Human endothelial colony forming cell	6	None
HEK293	Human embryonic kidney	3	8 µg/mL
HeLa	Human epithelial carcinoma	3	8 µg/mL
HT1080	Human fibrosarcoma	3	8 µg/mL
HUVEC	Human primary umbilical vein endothelial	6	None
MCF10a	Human mammary fibrocystic disease	3	3–8 µg/mL
MCF-7	Human mammary adenocarcinoma	3	3–8 µg/mL
MDA-MB-231	Human breast, adenocarcinoma	3	8 µg/mL
NIH-3T3	Mouse embryo fibroblast	6	8 µg/mL
SH-Sy5Y	Human brain neuroblastoma	3	4 µg/mL

Table 1: Recommended Polybrene® concentrations and MOI for common cell lines

Multiplicity of Infection (MOI)

The optimal MOI for your cells can be determined empirically in a 96-well plate.

1. Plate at least two densities of cells in a 96-well plate in appropriate medium.
   Note: Passage number can have a significant effect on lentiviral transduction efficiency. Low passage cells should be used in all experiments.
2. Incubate cells overnight in a 37° C, 5% CO_2 incubator.
3. Prepare transduction media containing lentivirus at a range of MOI plus appropriate concentration of Polybrene®.
4. Remove growth media and replace with transduction media.
5. After 24 hours, replace transduction media with growth media and return cells to incubator.
6. 48–72 hours after transduction, evaluate the efficiency of transduction by endpoint staining with the cell-permeable DNA dye Vybrant® DyeCycle™ Green at a final concentration of 1 µM (ThermoFisher).
7. Incubate at 37° C, 5% CO_2 incubator for 1 hour. After incubation, schedule a single scan in an Incucyte® Live-Cell Analysis System to acquire endpoint total DNA (Vybrant® DyeCycle™ Green stained) objects.



Example: Complete media containing 0.5 µg/mL puromycin or 40-100 µg/mL Bleomycin).

Licenses 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.

This Essen BioScience product 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 right to use Evrogen Fluorescent Proteins only for research conducted by the buyer. No rights are conveyed to modify or clone the gene encoding fluorescent protein contained in this product or to use Evrogen Fluorescent Proteins for commercial purposes. The right to use Evrogen Fluorescent Proteins specifically excludes the right to validate or screen compounds for commercial purposes. For information on commercial licensing, contact Evrogen Licensing Department, email: license@evrogen.com.

For Research Use Only. Not For Therapeutic or Diagnostic Use.

North America
Phone +1 734 769 1600, ext. 1
Email orders.US07@sartorius.com

Europe
Phone +44 (0) 1763 227400
Email euorders.UK03@sartorius.com

Japan
Phone +81 3 6478 5202
Email orders.US07@sartorius.com

China
Phone +86 21 6878 2300

Rest of Asia Pacific and other countries around the world:
Phone +65 6872 3966

For further contacts, visit
www.sartorius.com/incucyte

Incucyte-Cytolight-Lentivirus-Product-Guide-en-L-8000-0531-B00-Sartorius. Version 05 | 2021