SARTORIUS

Product Guide

Incucyte® Nuclight Lentivirus Reagents

For Nuclear Labeling of Live Cells

Product Information

Presentation, Storage and Stability
Incucyte® Nuclight Lentivirus Reagents are supplied as 0.2 mL or 0.6 mL vials of 3rd generation HIV-based, VSV-G pseudotyped lentiviral particles suspended in DMEM.

Product Name	Compatibility*	Amount	Cat. No.	Storage	Stability
Incucyte® Nuclight Green Lentivirus 2.0 (puro)	G | R	0.6 mL	BA-04888	-80° C	6 months from date of receipt
Incucyte® Nuclight Green Lentivirus 2.0 (bleo)	G | R	0.6 mL	BA-04890		
Incucyte® Nuclight Red Lentivirus 2.0 (puro)	G | R	0.6 mL	BA-04887		
Incucyte® Nuclight Red Lentivirus 2.0 (bleo)	G | R	0.6 mL	BA-04889		
Incucyte® Nuclight NIR Lentivirus (puro)	G | O | NIR or O | NIR	0.2 mL	4805		

G | R – Compatible with Incucyte® Live-Cell Analysis Systems configured with a Green | Red Optical Module
G | O | NIR or O | NIR – Compatible with Incucyte® Live-Cell Analysis Systems configured with Green | Orange | NIR or Orange | NIR Optical Module

Background

Incucyte® Nuclight Lentivirus Reagents enable efficient, non-perturbing, nuclear labeling of living mammalian cells. They are compatible with convenient transduction protocols and enable real-time cell counting and the calculation of cell doubling times. Incucyte® Nuclight Lentivirus provide homogenous expression of a nuclear-restricted mEGFP (green fluorescent protein), mCherry2 (red fluorescent protein) or iRFP713 (NIR fluorescent protein) driven by EF-1α promoter in your choice of primary, immortalized, dividing, or non-dividing cells. These reagents are ideal for generating stable cell populations or clones using puromycin or bleomycin selection.

Recommended Use

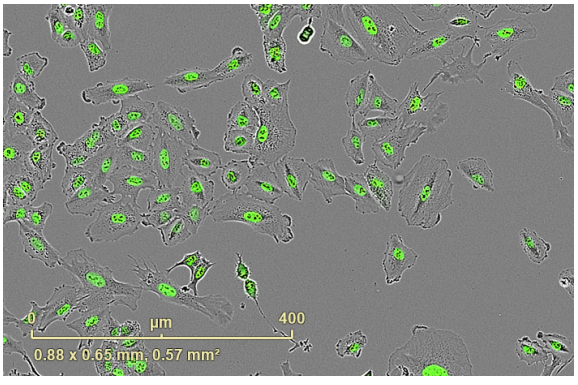
We recommend thawing the Incucyte® Nuclight Lentivirus on ice and storing working aliquots at -80 °C, as excessive freeze | thaw cycles can impair transduction efficiency. The lentivirus reagents can be prepared in full media and added directly to plated cells. We recommend an MOI of 3 to 6, dependent on the cell type being infected. The cationic polymer Polybrene® may be added to enhance transduction efficiency. Post infection, stable cells may be generated using appropriate antibiotic selection.

For viral titer and lot information please visit our web page **https://www.sartorius.com/en/products/live-cell-imaging-analysis/live-cell-analysis-reagent-consumables/lentivirus-viral-titers**

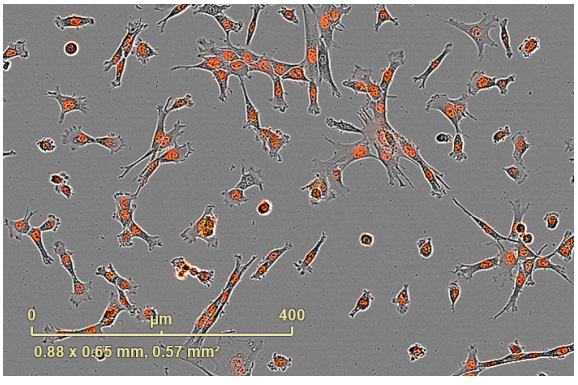
Safety data sheet (SDS) information can be found on our website at **www.sartorius.com/incucyte-shop**

Example Data

A549 Nuclight Green



NIH-3T3 Nuclight Red



U87 Nuclight NIR

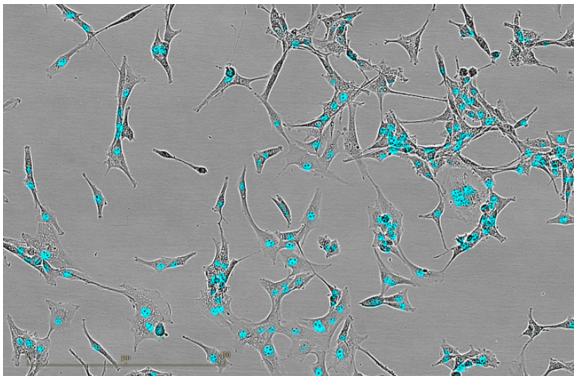


Figure 1: Representative images of A549, NIH-3T3, and U87 cells transduced with the Incucyte® Nuclight Lentivirus Reagents. Note the nuclear restricted expression of green (mEGFP), red (mCherry2), or NIR fluorescent protein (iRFP713) and the healthy cell morphology.

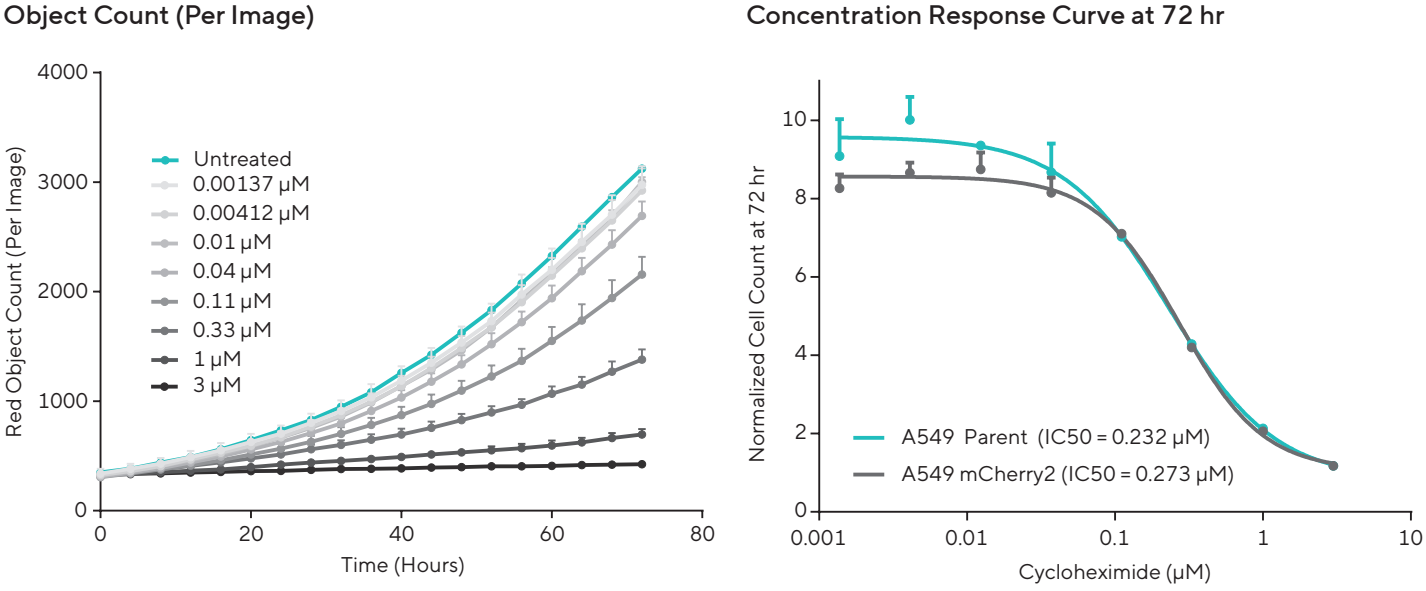
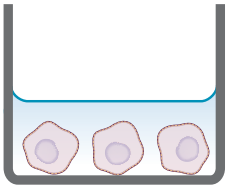


Figure 2: Concentration-dependent inhibition of proliferation by the protein synthesis inhibitor cycloheximide in A549 adenocarcinomic epithelial cells labeled with the Incucyte® Nuclight Red Lentivirus 2.0. A) Time-course of nuclear count in the absence (teal color) and increasing concentrations of cycloheximide (progressively darker symbols). B) Concentration response curve to cycloheximide. Cell counts at 72 h have been determined from the time-course shown in panel (A) and compared with uninfected A549 control cells revealing equivalent pharmacology between Incucyte® Nuclight Red 2.0 labeled and uninfected cells.

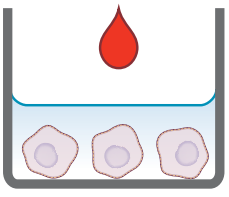
Quick Guide

1. Seed cells



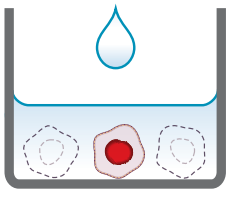
Seed cells in growth media and leave to adhere (4–24 hours). Cells should be 15–35% confluent at the time of transduction.

2. Transduce



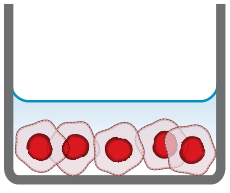
Add Incucyte® Nuclight Lentivirus (MOI 3 to 6) diluted in media ± Polybrene®. After 24 hours, replace the media with fresh growth media. Monitor expression using the Incucyte® Live-Cell Analysis System.

3. Apply selection



Apply antibiotic selection to derive a stable, homogenous cell population or clone that expresses a nuclear restricted fluorescent protein. (Optional: Freeze cells and use for future assays).

4. Live-cell fluorescent imaging



Capture images according to assay requirements (e.g., every 1–12 hours) in an Incucyte® Live-Cell Analysis System. Analyze using integrated software.

Protocols and Procedures

Materials

Required Materials

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

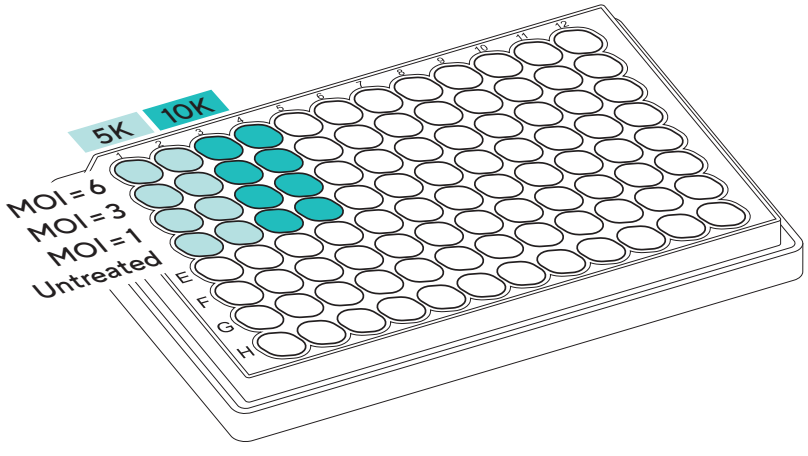
Optional

- 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® Nuclight Lentivirus Reagents 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.
2. Add Incucyte® Nuclight 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 (Table 1).
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 or bleomycin) at the concentration determined from the kill curve (see next page "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.



Example: Complete media containing 0.5 µg/mL Puromycin or 40 – 100 µg/mL Bleomycin).

Suggested Infection Protocol for Primary Cells and Transient Assays

If you do not plan to use the Incucyte® Nuclight Lentivirus Reagents to create stably expressing cells, then 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 (for Stable Protein Expression in Immortalized Cell Lines)

To determine the lowest concentration of antibiotic selection required to efficiently eliminate non-transduced cells, perform a kill curve using several concentrations of the relevant selection antibiotic for your Incucyte® Nuclight Lentivirus (i.e., puromycin or bleomycin).

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 ± 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 infection, evaluate the efficiency of transduction by end-point staining with a cell-permeable DNA dye such as 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 nuclear counts (e.g., Vybrant® DyeCycle™ Green stained objects).

Polybrene® Concentration

The cationic polymer, Polybrene®, may be used to increase the efficiency of transduction. Optimal Polybrene® concentrations will vary depending on the cell type used. The following table provides recommended transduction conditions for several common cell lines. 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 Concentration
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
HT 1080	Human fibrosarcoma	3	8 µg/mL
HUVEC	Human primary umbilical vein endothelial	6	None
MCF10a	Human mammary fibrocystic disease	3	3 – 8 µg/mL
MCF7	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

Safety Considerations

Sartorius products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling lentivirus reagents. Please read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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 (U_3) 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.
- 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 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.

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

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Straße 20
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Corporation
3874 Research Park Drive
Ann Arbor, MI 48108
Phone +1 734 769 1600

Orders EMEA
Email: orderhandling.lps.ne@sartorius.com

Orders USA
Email: LPS.OPM.NA@sartorius.com

Find out more: sartorius.com/incucyte

For questions, email: AskAScientist@sartorius.com

Status: 03 | 2025