IncuCyte® NucLight Lentivirus Reagents for Nuclear Labeling

Create stable cell populations or clones expressing a nuclear restricted fluorescent label.

Product Information

Presentation, storage and stability

IncuCyte NucLight 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 NucLight Lentivirus Reagents will be stable for at least 3 months from the date of receipt.

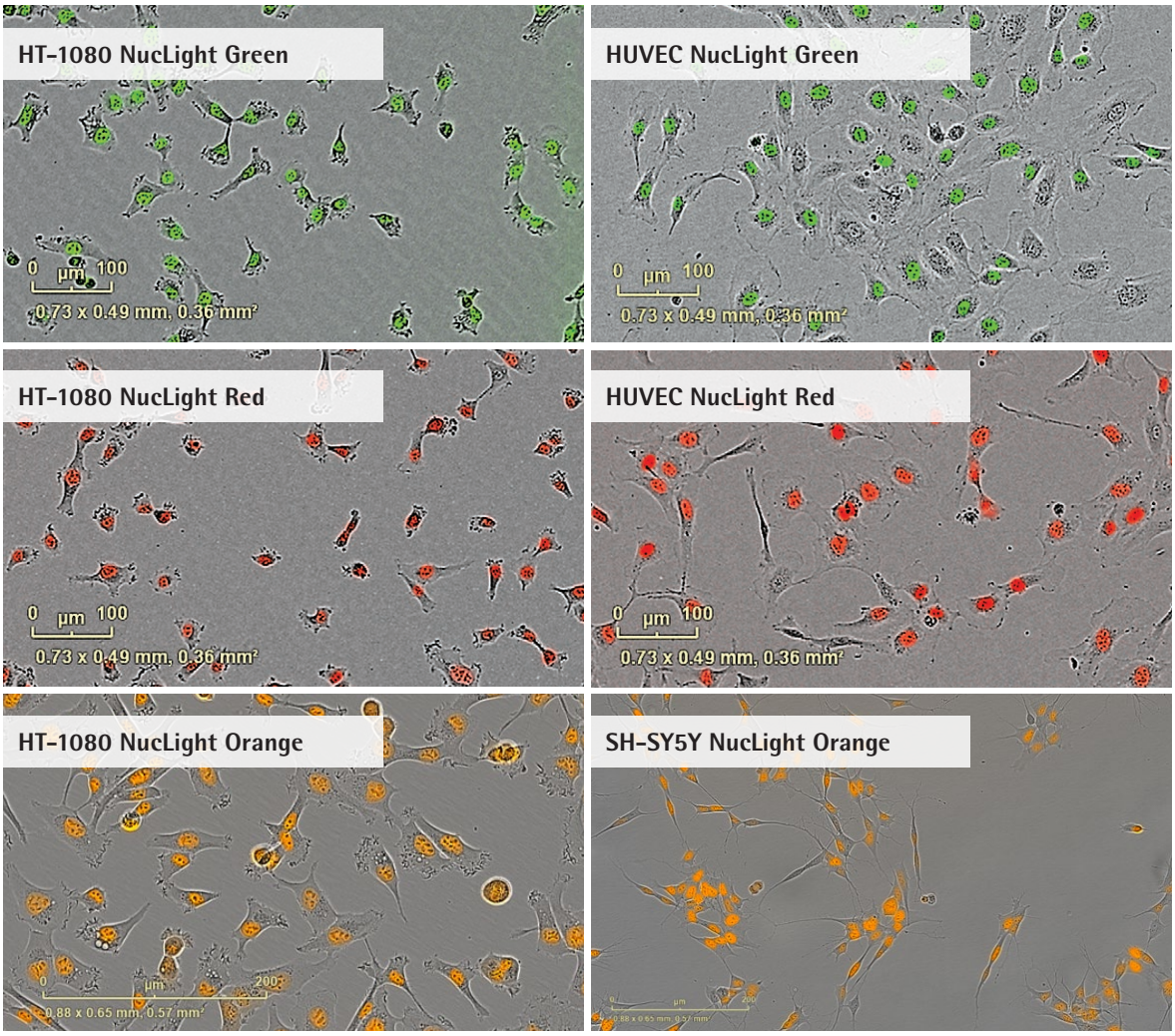
Background and intended use

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 Reagents provide homogenous expression of a nuclear-restricted GFP (green fluorescent protein), mKate2 (red fluorescent protein), or TagRFP (orange 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 or bleomycin selection. The IncuCyte NucLight Lentivirus Reagents have been validated for use with the IncuCyte® Live-Cell Analysis System. Please see table on page 3 for reagent information and instrument compatibility.

Recommended use

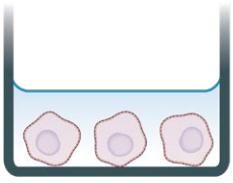
We recommend that the IncuCyte NucLight Lentivirus Reagents are thawed on ice and working aliquots are stored at -80° C. Excessive freeze/thaw cycles can impair transduction efficiency. The lentivirus reagents can be prepared in full media and added directly to plated cells for transduction. We recommend an MOI of 3 to 6 dependent 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 proliferation assays.

Figure 1. Representative images of primary (HUVEC) and tumor (HT-1080) and MDA-MB-231 cells transduced with the IncuCyte NucLight Lentivirus Reagents. Note the nuclear restricted expression of red (mKate2), orange (TagRFP), green fluorescent protein (GFP), and the healthy cell morphology.



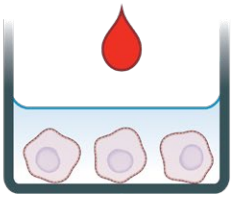
Quick guide

1. Seed cells



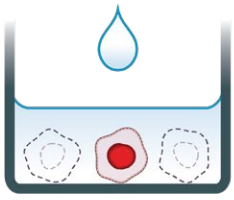
Cell Seeding
Seed cells in growth media and leave to adhere (4-24 hours). Cells should be 15-35% confluent at the time of transduction.

2. Transduce



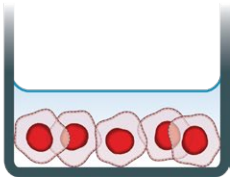
Add IncuCyte NucLight Lentivirus Reagent
Add NucLight Lentivirus Reagent (MOI 3 to 6) diluted in media ± Polybrene®. After 24 hours, replace the media with fresh growth media. Monitor expression using the IncuCyte Live-Cell Analysis System.

3. Apply selection



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

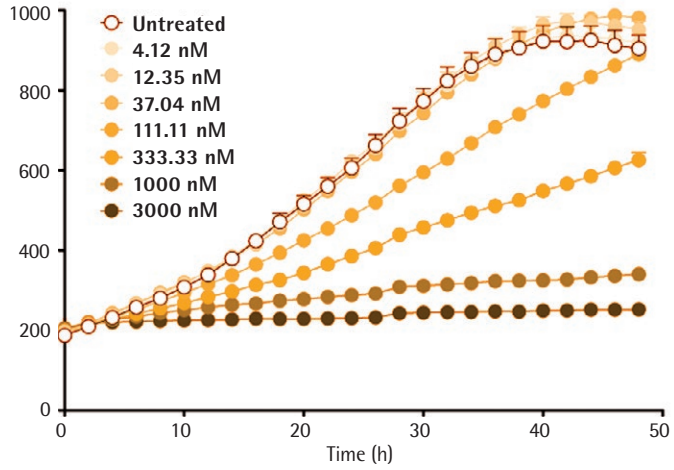
4. Live-cell fluorescent imaging



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.

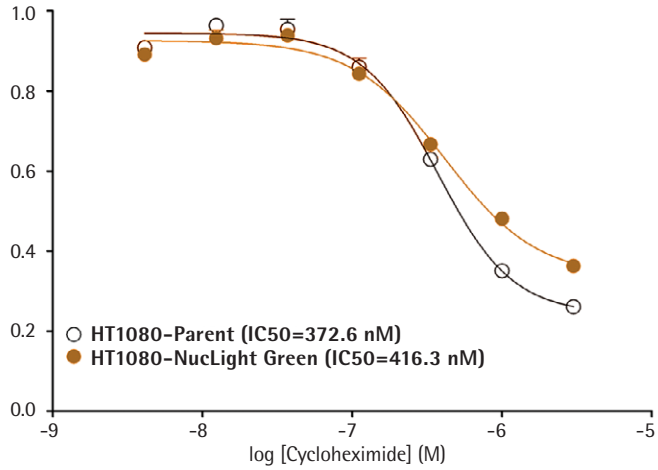
Figure 2. Concentration-dependent inhibition of proliferation by the protein biosynthesis inhibitor cycloheximide in HT-1080 fibrosarcoma cells labelled with the IncuCyte® NucLight™ Green Lentivirus Reagent.

A. Green Object Count (per mm²)



(A) Time-course of nuclear count in the absence (open symbols) and increasing concentrations of cycloheximide (progressively darker symbols).

B. Normalized Cell Count at 48 h



(B) Concentration response curve to cycloheximide. Cell counts at 48 h have been determined from the time-course shown in panel A and compared to uninfected HT-1080 control cells revealing equivalent pharmacology between IncuCyte NucLight Green Lentivirus labeled and uninfected cells.

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Product					Instrument Compatibility
IncuCyte® NucLight Red Lentivirus Reagent (EF-1∝, Puro)	**Cat. No.:**	4625	**Ex. Maxim:**	588 nm	IncuCyte Live-Cell Analysis System (Green/Red)
	Promoter:	EF-1∝	**Em. Maxima:**	633 nm	
	Amount:	0.2 mL			
IncuCyte® NucLight Red Lentivirus Reagent (EF-1∝, Puro)	**Cat. No.:**	4476	**Ex. Maxim:**	588 nm	IncuCyte Live-Cell Analysis System (Green/Red)
	Promoter:	EF-1∝	**Em. Maxima:**	633 nm	
	Amount:	0.6 mL			
IncuCyte® NucLight Red Lentivirus Reagent (EF-1∝, Bleo)	**Cat. No.:**	4627	**Ex. Maxim:**	588 nm	IncuCyte Live-Cell Analysis System (Green/Red)
	Promoter:	EF-1∝	**Em. Maxima:**	633 nm	
	Amount:	0.2 mL			
IncuCyte® NucLight Red Lentivirus Reagent (EF-1∝, Bleo)	**Cat. No.:**	4478	**Ex. Maxim:**	588 nm	IncuCyte Live-Cell Analysis System (Green/Red)
	Promoter:	EF-1∝	**Em. Maxima:**	633 nm	
	Amount:	0.6 mL			
IncuCyte® NucLight Green Lentivirus Reagent (EF-1∝, Puro)	**Cat. No.:**	4624	**Ex. Maxim:**	483 nm	IncuCyte Live-Cell Analysis System (Green/Red)
	Promoter:	EF-1∝	**Em. Maxima:**	506 nm	
	Amount:	0.2 mL			
IncuCyte® NucLight Green Lentivirus Reagent (EF-1∝, Puro)	**Cat. No.:**	4475	**Ex. Maxim:**	483 nm	IncuCyte Live-Cell Analysis System (Green/Red)
	Promoter:	EF-1∝	**Em. Maxima:**	506 nm	
	Amount:	0.6 mL			
IncuCyte® NucLight Green Lentivirus Reagent (EF-1∝, Bleo)	**Cat. No.:**	4626	**Ex. Maxim:**	483 nm	IncuCyte Live-Cell Analysis System (Green/Red)
	Promoter:	EF-1∝	**Em. Maxima:**	506 nm	
	Amount:	0.2 mL			
IncuCyte® NucLight Green Lentivirus Reagent (EF-1∝, Bleo)	**Cat. No.:**	4477	**Ex. Maxim:**	483 nm	IncuCyte Live-Cell Analysis System (Green/Red)
	Promoter:	EF-1∝	**Em. Maxima:**	506 nm	
	Amount:	0.6 mL			
IncuCyte® NucLight Orange Lentivirus Reagent (EF-1∝, Puro)	**Cat. No.:**	4771	**Ex. Maxim:**	555 nm	IncuCyte Live-Cell Analysis System for Neuroscience (Orange/NIR)
	Promoter:	EF-1∝	**Em. Maxima:**	584 nm	
	Amount:	0.2 mL			

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

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 to the plating surface.
2. Add IncuCyte NucLight Lentivirus Reagent 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 or zeocin) at the concentration determined from the kill curve (see next section "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 (optional)

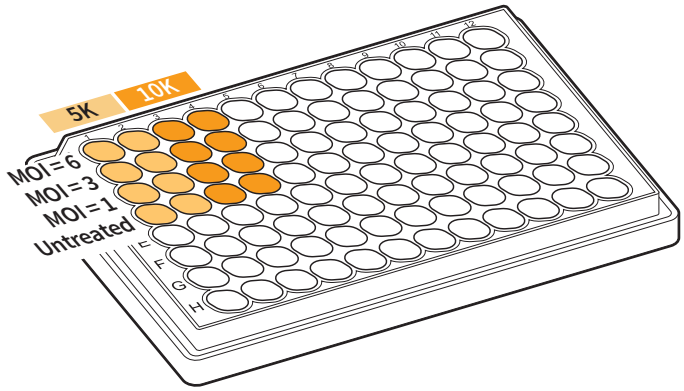
To determine the lowest concentration of antibiotic selection required to efficiently eliminate non-transduced cells, perform a kill curve using several concentrations of the relevant selection marker for your IncuCyte NucLight Lentivirus Reagent (i.e., puromycin or bleomycin).

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.

Recommended Polybrene® Concentrations and MOI for Common Cell Lines

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
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



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 end-point staining with the 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 DNA (e.g. Vybrant® DyeCycle™ Green stained) objects.

A complete suite of cell health applications is available to fit your experimental needs. Find more information at **www.sartorius.com/incucyte**

For additional product or technical information, please e-mail us at **AskAScientist@sartorius.com** or visit our website at **www.sartorius.com/incucyte**

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