SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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IncuCyte® NucLight Lentivirus Reagents for Nuclear Labeling

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

Presentation, storage and stability

IncuCyte NucLight Lentivrus 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 labelling 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) 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 or bleomycin selection. The IncuCyte NucLight Lentivirus Reagents have been validated for use with the IncuCyte® live-cell analysis system and can be combined with the IncuCyte® Caspase 3/7 Reagent or IncuCyte® Cytotox Reagents for multiplexed measurements of apoptosis and cytotoxicity alongside cell proliferation in every well.

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. Please see the relevant protocol published on our website: **essenbioscience.com/nuclight**

Safety data sheet (SDS) information

The SDS can be found on our website: **essenbioscience.com/nuclight**

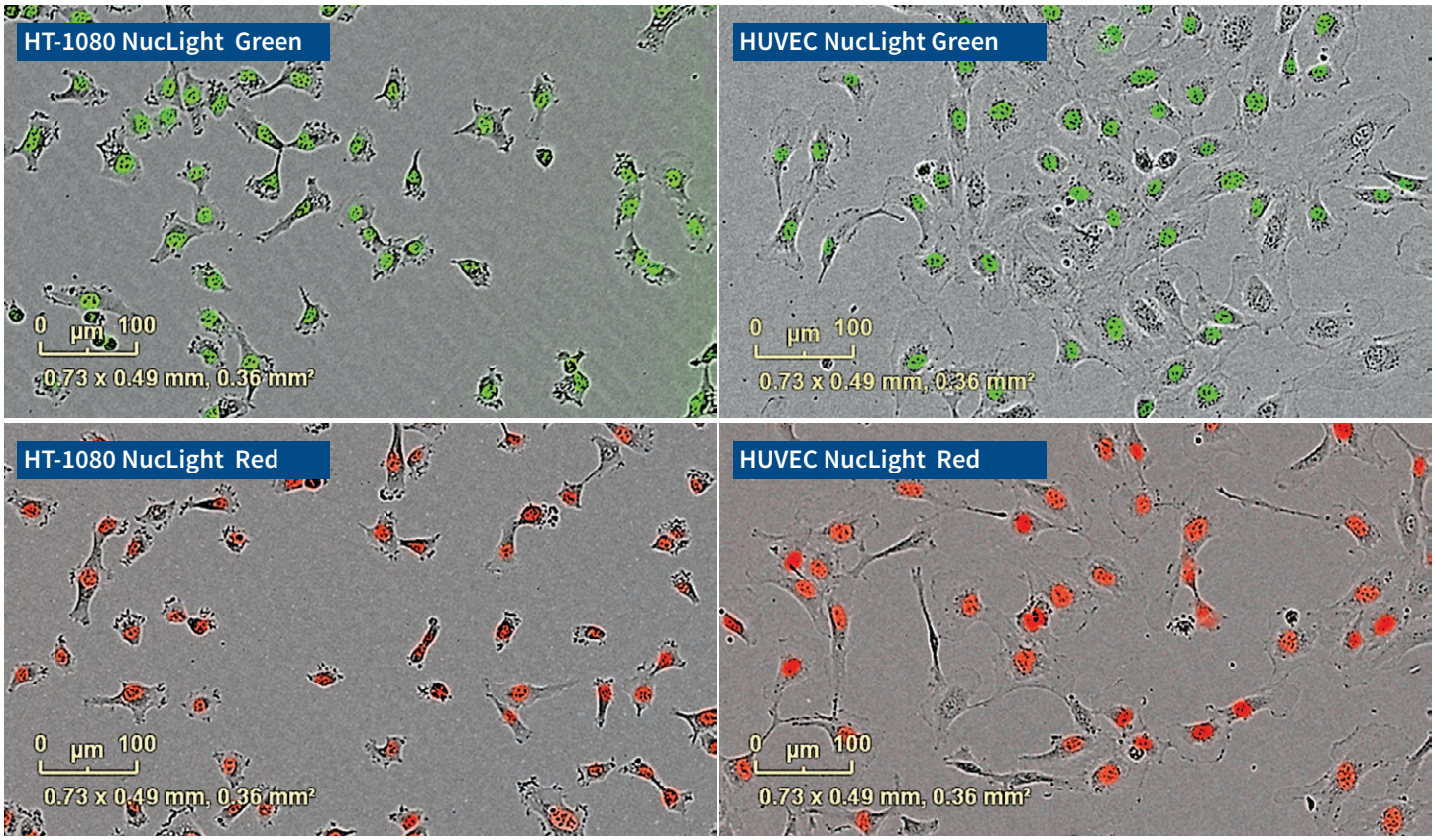
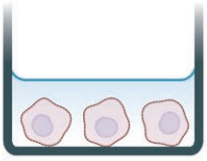


Figure 1. Representative images of primary **(HUVEC)** and tumor **(HT-1080)** cells transduced with the IncuCyte NucLight Lentivirus Reagents. Note the nuclear restricted expression of red (mKate2) or green fluorescent protein (GFP) and the healthy cell morphology.

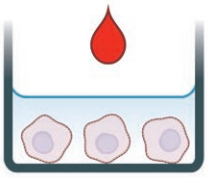
Quick guide

1 SEED CELLS



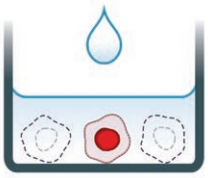
Cell Seeding
Seed cells in growth media and leave to adhere (4-24 hours). Cells should be 15-35% confluent at the time of transduction.

2 TRANSDUCE



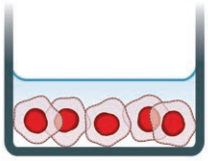
Add Incucyte Nuclight Lentivirus Reagent
Add Green or Red NucLight Lentivirus Reagent (MOI 3 to 6) diluted in media ± Polybrene®. After 24 hours, replace the media with fresh growth media. Monitor expression using the IncuCyte live-cell analysis system.

3 APPLY SELECTION



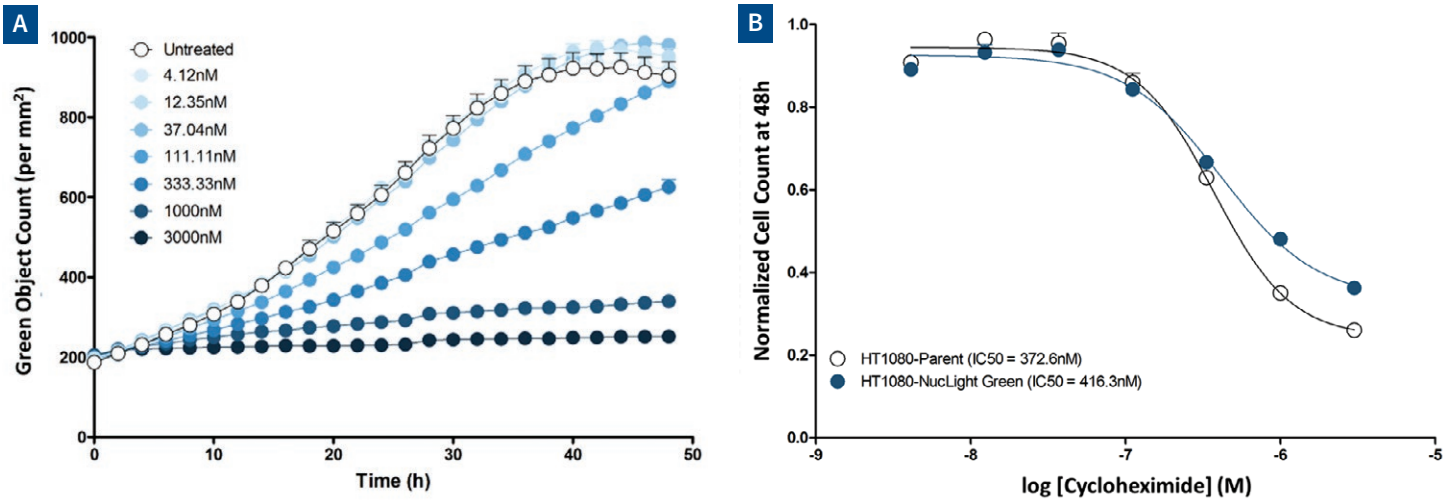
Generate a Stable Population or Clone
Apply antibiotic selection to derive a stable, homogenous cell population or clone that expresses a nuclear restricted green or red fluorescent protein. (Optional: Freeze cells and use for future assays).

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

(B) Concentration response curve to cycloheximide. Cell counts at 48h 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.

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE.

Product	Cat No.	Promoter	Amount	Ex. maxima	Em. maxima
IncuCyte® NucLight Red Lentivirus Reagent (EF-1α, Puro)	4625	EF-1α	0.2mL	588 nM	633 nM
IncuCyte® NucLight Red Lentivirus Reagent (EF-1α, Puro)	4476	EF-1α	0.6mL	588 nM	633 nM
IncuCyte® NucLight Red Lentivirus Reagent (EF-1α, Bleo)	4627	EF-1α	0.2mL	588 nM	633 nM
IncuCyte® NucLight Red Lentivirus Reagent (EF-1α, Bleo)	4478	EF-1α	0.6mL	588 nM	633 nM
IncuCyte® NucLight Green Lentivirus Reagent (EF-1α, Puro)	4624	EF-1α	0.2mL	483 nM	506 nM
IncuCyte® NucLight Green Lentivirus Reagent (EF-1α, Puro)	4475	EF-1α	0.6mL	483 nM	506 nM
IncuCyte® NucLight Green Lentivirus Reagent (EF-1α, Bleo)	4626	EF-1α	0.2mL	483 nM	506 nM
IncuCyte® NucLight Green Lentivirus Reagent (EF-1α, Bleo)	4477	EF-1α	0.6mL	483 nM	506 nM

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

Protocols and Procedures

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 section below, "Optimizing 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 Zeocin).

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.

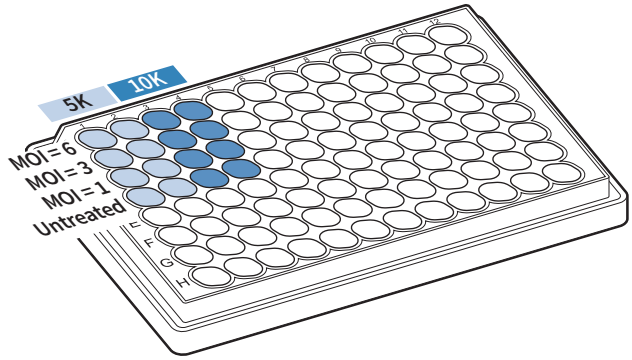
Recommended Polybrene® Concentrations and MOI for Common Cell Lines

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
MSA-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 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.

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").

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