

CellPlayer™ CytoLight Red (Lenti, EF-1 alpha, puro)

Essen BioScience Catalog Number: 4482

Background

Third generation lentiviral-based vectors are commonly used to transfer genetic information to cells for gene therapy and/or research purposes. The Essen BioScience CellPlayer™ lentiviral-based reagents have been specially designed to efficiently transduce multiple cell types and provide homogenous expression of fluorescent protein across a population of primary or immortalized, dividing or non-dividing cells with low toxicity. Our extensive validation experiments have shown that expression of nuclear restricted RFP does not negatively alter functional cell biology (e.g. morphology, proliferation, migration, and differentiation). These reagents can be used either transiently or to generate stable cell populations or clones using puromycin selection. The Essen CellPlayer™ lentiviral-based fluorescent protein reagents are particularly suited for use with the InCuCyte ZOOM™ live-cell imaging system.

Virus Description

3rd generation HIV-based, VSV-G pseudotyped lentiviral particles encoding a Red Fluorescent Protein.

Promoter: EF-1 alpha

Selectable Marker: Puromycin

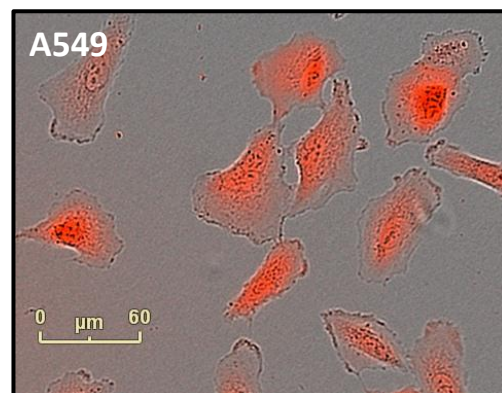
Spectral Properties: Ex (max): 588 nm; Em (max): 633 nm

Presentation

Lot #:

Viral Titer:

Volume: 0.6 mL



Storage

Lentivirus is stable for at least 3 months from date of receipt when stored at -80°C. After thawing, place immediately on ice and freeze in working aliquots at -80°C. **Additional freeze/thaw cycles may result in decreased viral titers and sub-optimal transduction efficiencies.**

Additional Materials

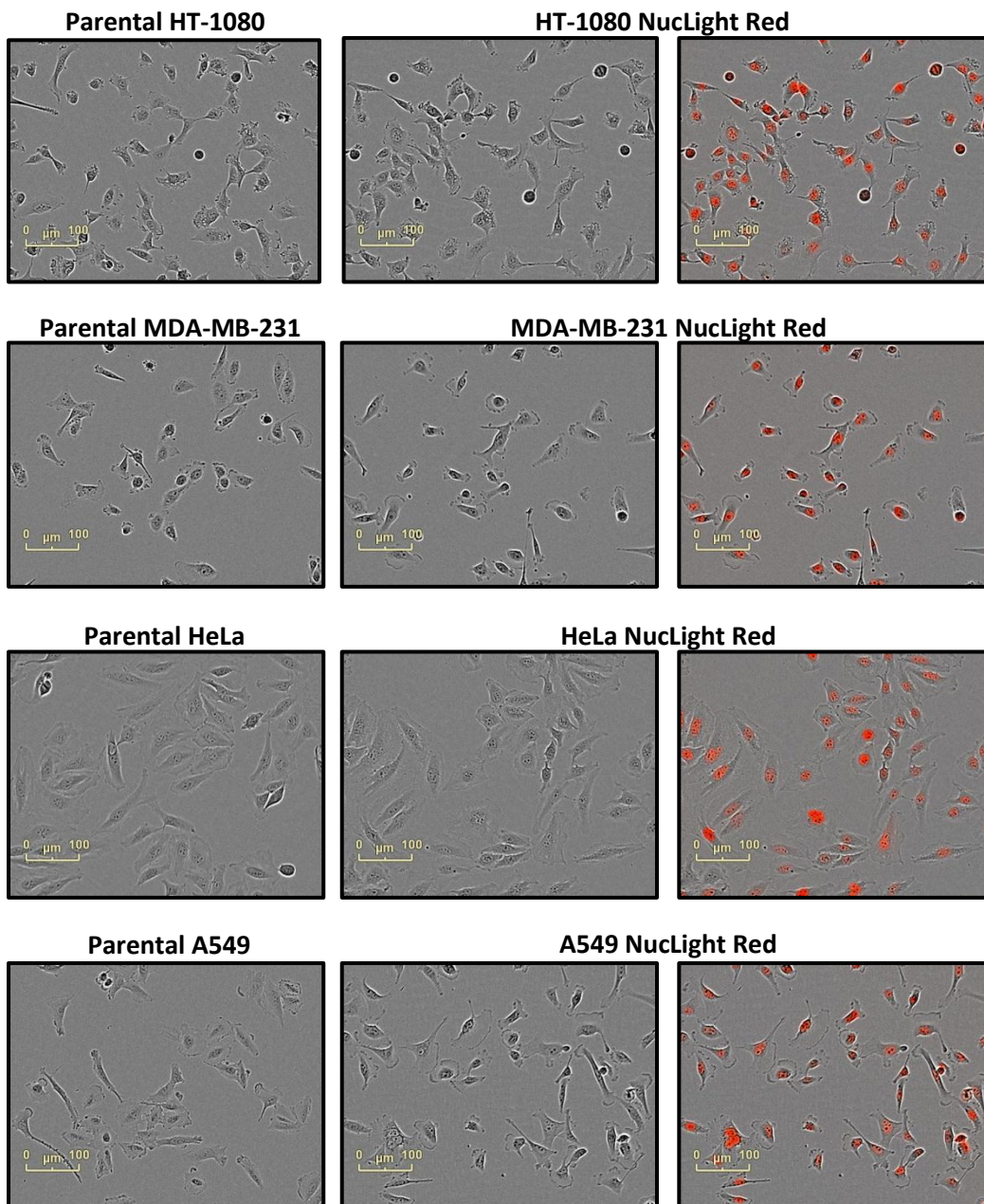
(Optional) Hexadimethrine Bromide; aka Polybrene® (2 mg/mL stock solution; Sigma-Aldrich: H9268)



Validation Assays (Quality Control Testing)

The following experiments were completed using an IncuCyte ZOOM™ (10x) using NuLight Red expressing cells.

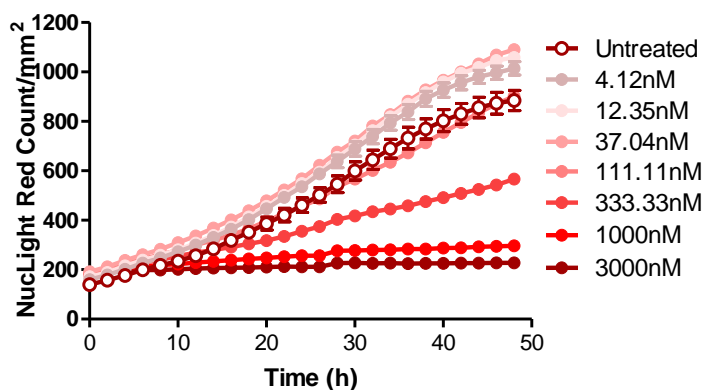
1. Morphological Comparison – No morphological differences between transduced and parental populations.



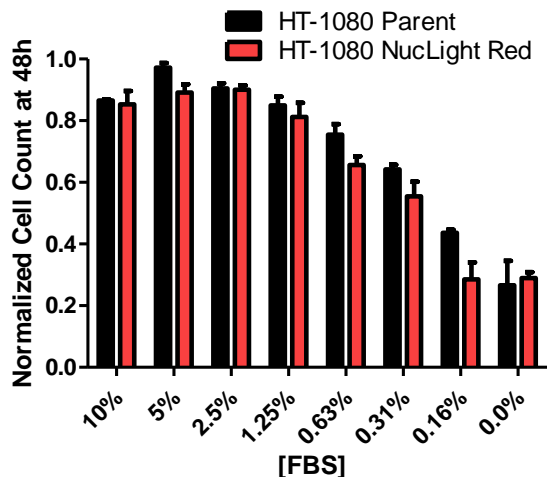
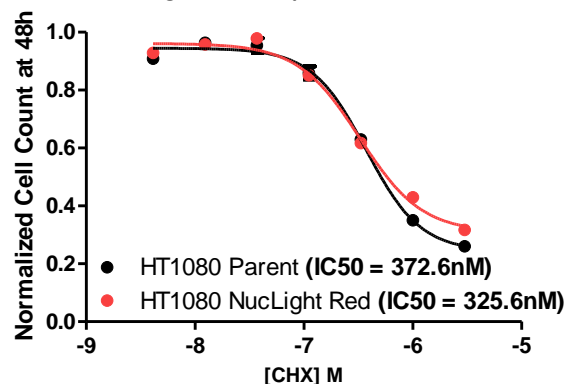


2. Proliferation

Real-Time Cell Counts of HT-1080 NuLight Red cells
Treated with Cycloheximide



Cycloheximide Pharmacology: HT-1080
NuLight Red Compared to Parent Cells



Cell Type	Proliferation (CHX IC50)	
	Parent	NuLight Red
HT-1080	372.6 nM	325.6 nM
HeLa	560.4 nM	979.1 nM
MDA-MB-231	335.8 nM	602.2 nM
A549	181.6 nM	246 nM

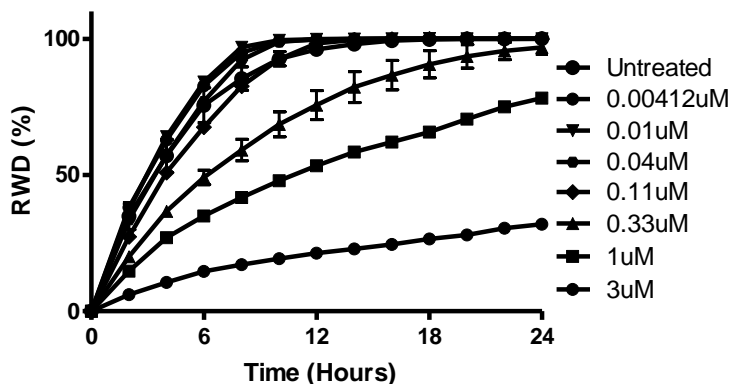
*Raw data can be found in individual Data Sheets for each cell line

Results: Each of the cell lines listed above (HT-1080, MDA-MB-231, HeLa, and A549) have been extensively analyzed to determine if infection of Lentivirus or expression of NuLight Red nuclear label has a detrimental effect on cell proliferation. The kinetic graph (Top Left) illustrates the concentration response of HT-1080 NuLight Red cells to cycloheximide treatment. At the 48 hour endpoint, identically treated parental controls were stained with Vybrant DyeCycle Green and counted. Pharmacological analysis at the endpoint revealed similar cycloheximide IC50 concentrations for both parent and NuLight populations (Top Right). This analysis did not reveal a substantial shift in pharmacology in stable NuLight Red populations compared to identically treated parental cells (Table). Each cell type was also grown in reduced serum conditions (Bottom Left). Again, no differences in growth characteristics were observed between parent populations and stable populations expressing NuLight Red (raw data can be found in Product Data Sheets for each cell type). Together these data indicate that Lentivirus transduction and NuLight Red expression do not alter proliferation of cells relative to the parental controls.

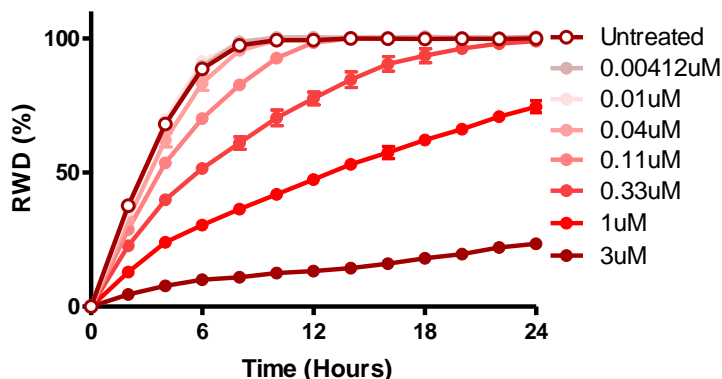


3. Cell Migration

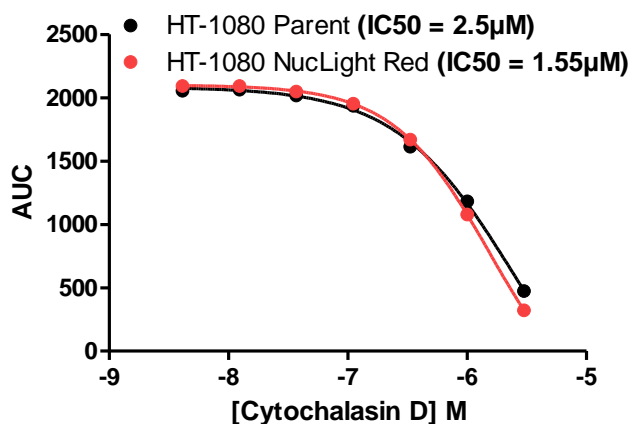
Kinetic Pharmacology: Cytochalasin D Treated Parental HT-1080 Cells



Kinetic Pharmacology: Cytochalasin D treated HT-1080 NuLight Red cells



Kinetic Pharmacology: AUC Analysis and IC50 Calculation



Cell Type	Migration (CytoD IC50)	
	Parent	NuLight Red
HT-1080	2.5 µM	1.55 µM
HeLa	640 nM	229 nM
MDA-MB-231	81.9 nM	84.7 nM
A549	100.7 nM	93.7 nM

*Raw data can be found in individual Data Sheets for each cell line

Results: The migration kinetics of each cell type was also analyzed. As an example, parental HT-1080s and the stable HT-1080 NuLight Red population were evaluated using the label-free Essen CellPlayer 96-well Cell Migration assay in conjunction with the Essen WoundMaker tool (Cat# 4443). Cells were treated with decreasing concentrations of cytochalasin D, a potent inhibitor of actin polymerization. Concentration dependent inhibition of wound closure, analyzed using Essen's Relative Wound Density (RWD) metric, was observed in both parental and stable HT-1080 NuLight Red cells at concentrations of CytoD $\geq 0.33 \mu\text{M}$. Pharmacological analysis using the area under the curve (AUC) of the kinetic traces revealed similar IC50 values for cytochalasin D treatment. Summary statistics for each cell type can be found in the associated Table.



Protocols and Procedures

General Infection Protocol

1. Seed cells in growth media of choice at a density such that they are 15-35% confluent at time of infection. Incubate 24 hours, or enough time for cells to attach to plating surface.
Example: Seed 10,000 HT-1080 cells in one well of a 24-well plate
2. Add Lentivirus at desired multiplicity of infection (MOI = TU/cell). An MOI of 3 is recommended for most cell types. However, an optimized MOI should be determined for each cell type in use, especially for transient assays. Polybrene® (1-8 µg/mL) can be used to enhance transduction of many cell types (Note: Some cell types are sensitive to Polybrene® (e.g. neurons).
*Example: $10,000 \times 3 \text{ TU/cell (MOI)} = 30,000 \text{ TU}$; $30,000 \text{ TU} \div 1.57 \times 10^6 \text{ TU/mL} = 0.0191 \text{ mL}$ or $19.1 \mu\text{l}$.
Transduction of HT-1080 cells can be greatly enhanced if transduced in the presence of Polybrene®.
Recommended Polybrene concentrations range from 1-8 µg/mL depending on the cell type. For HT-1080 cells, 8 µg/mL Polybrene® is recommended.*
3. Incubate at 37°C, 5% CO₂ for 24 hours.
4. Remove media and replace with fresh growth media.
NOTE: Media should be treated as biohazardous waste and treated with a 10% bleach solution prior to disposal per waste disposal guidelines.
5. Return to incubator for an additional 24-48 hours, monitoring expression using an IncuCyte ZOOM™ or fluorescence microscope.
6. Pick up cells and distribute at desired density for experiment. For stable selection, proceed to step 7.
7. (Optional) Remove media and replace with fresh growth media containing Puromycin selection.
Example: For HT-1080, A549, HeLa, and MDA-MB-231 cells, media containing 1 µg/mL Puromycin is sufficient for efficient killing of non-transduced cells.
8. Incubate for 72-96 hours, replacing media every 48 hours.
9. Maintain stable population in a maintenance concentration of selection media.
Example: HT-1080, A549, HeLa, and MDA-MB-231 CytoLight Red cells can be maintained in complete media containing 0.5 µg/mL Puromycin.



Optimizing Polybrene® Concentration

Optimal Polybrene® concentrations will vary depending on cell type. The following table provides transduction conditions for several common cell lines from both Essen's experience and reported from other sources. Please note, Polybrene® can be toxic to certain cell types (e.g. primary neurons). The standard Essen CellPlayer Cytotoxicity protocol can be used to evaluate the toxic effect of Polybrene® on your cells.

1. Plate cells at a range of cell densities and culture overnight.
2. Replace the culture medium with fresh medium containing a range of Polybrene® concentrations (0-8 µg/mL) in the presence of YOYO-1 (optional, recommended concentration 100 nM) and return to incubator overnight.
3. Monitor cells for loss of membrane integrity (or other marker of cell death), and examine culture for cell viability. Identify the highest concentration of Polybrene® that does not cause toxicity.

Use this optimized concentration of Polybrene® for subsequent optimization steps.

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

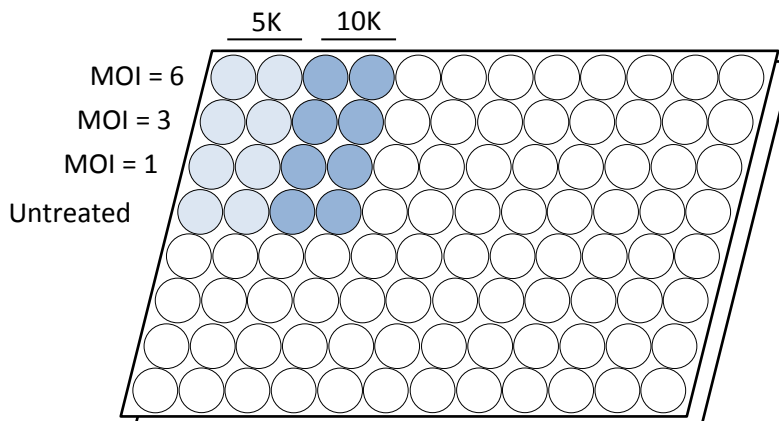


Optimizing Multiplicity of Infection (MOI)

Determining the optimal MOI for your cell line can be completed 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% CO₂ incubator
3. Prepare transduction media plus appropriate concentration of Polybrene® and replace growth media with transduction media.
4. After 24 hours, replace transduction media with growth media and return cells to incubator.
5. 48-72 hours after transduction, evaluate cells for efficiency of transduction:

Example: If using NuLight Red Lenti, stain cells with Vybrant DyeCycle Green following our standard no-wash protocol (Final concentration of 1 µM). Using the IncuCyte ZOOM™, count the number of objects prior to staining, then count the number of objects post stain. From these values, calculate transduction efficiency for each MOI. If using CytoLight Red Lenti, quantifying accurate transduction efficiencies can be a challenge as cells can be difficult to accurately mask. In this case, a qualitative measure may be more appropriate. If accurate transduction efficiencies are required, a flow cytometry approach may be more appropriate.

Optimizing Antibiotic Selection:

If the generation of stable populations or clones is the primary objective, optimization of MOI and transduction conditions is less important as the selection process will eliminate non- or low-expressing cells within the population. However, a kill curve with several concentrations of Puromycin should be completed on non-transduced cells to determine the lowest concentration of selection that is required to efficiently eliminate non-transduced cells.



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 (Δ 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 place 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, refer to 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>. You may also refer to the NIH's Lentivirus containment guidelines at:

http://oba.od.nih.gov/oba/rac/Guidance/LentiVirus_Containment/pdf/Lenti_Containment_Guidance.pdf

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.

Related Products

NuLight/CytoLight Reagents:

Cat.# 4475 CellPlayer NuLight Green (Lenti, EF-1 alpha, puro)
Cat.# 4481 CellPlayer CytoLight Green (Lenti, EF-1 alpha, puro)
Cat.# 4513 CellPlayer CytoLight Green (Lenti, CMV, no selection)

Cat.# 4476 CellPlayer NuLight Red (Lenti, EF-1 alpha, puro)
Cat.# 4482 CellPlayer CytoLight Red (Lenti, EF-1 alpha, puro)

NuLight Cell Lines:

Cat.# 4485 CellPlayer HT-1080 NuLight Red
Cat.# 4487 CellPlayer MDA-MB-231 NuLight Red
Cat.# 4489 CellPlayer HeLa NuLight Red
Cat.# 4491 CellPlayer A549 NuLight Red
Cat.# 4506 CellPlayer HUVEC NuLight Green
Cat.# 4453 CellPlayer HUVEC CytoLight Green

Cat.# 4486 CellPlayer HT-1080 NuLight Green
Cat.# 4488 CellPlayer MDA-MB-231 NuLight Green
Cat.# 4490 CellPlayer HeLa NuLight Green
Cat.# 4492 CellPlayer A549 NuLight Green
Cat.# 4511 CellPlayer Neuro-2a NuLight Green
Cat.# 4512 CellPlayer Neuro-2a NuLight Red





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