

CellPlayer™ A549 NuLight Red

Essen BioScience Catalog Number: 4491

Storage

Liquid Nitrogen

Note: Cells can be thawed and cultured immediately upon receipt or stored in liquid nitrogen for long-term storage. Storage at -80°C is not recommended. Cells should be used within 1 year of delivery.

Presentation

1mL, 1×10^6 cells/mL in 90% FBS, 10% DMSO

Recommended Media and Components

Ham's F-12 + GlutaMAX™-1 (Cat# 31765 Gibco/Life Technologies; Low riboflavin media for fluorescence imaging)

10% FBS (Cat# SH30071 Thermo Hyclone)

1% Pen/Strep (Cat# 15140 Gibco/Life Technologies)

0.5µg/ml Puromycin (Cat# A11138-03 Gibco/Life Technologies)

Background

Each vial contains a stable population of 1 million A549 cells expressing the NuLight Red fluorescent protein restricted to the nucleus. Parental A549 cells were purchased from ATCC (Cat# CCL-185). A549 cells were transduced with the Essen CellPlayer NuLight Red Lentivirus (Cat# 4476; EF1α, puromycin) at an MOI of 3 (TU/cell) in the presence of 8µg/ml polybrene following the standard Essen protocol. This resulted in ≥70% transduction efficiency. 48 hours post infection, the complete population of cells were grown for 3-5 days in complete growth media containing 1µg/ml Puromycin to select for cells expressing NuLight Red. NuLight Red expressing cells are maintained in complete media containing 0.5µg/ml Puromycin. Following selection, a panel of validation assays designed to evaluate the effects of nuclear label expression on functional cell biology was completed. These assays include comparisons of cell morphology, growth/proliferation, migration, and cytotoxicity between stable populations and the parent populations from which they were derived (see below). In addition, all cells in our NuLight Red catalog has been certified mycoplasma free by ATCC and our stable populations have been unambiguously authenticated using ATCC's Short Tandem Repeat (STR) profiling.

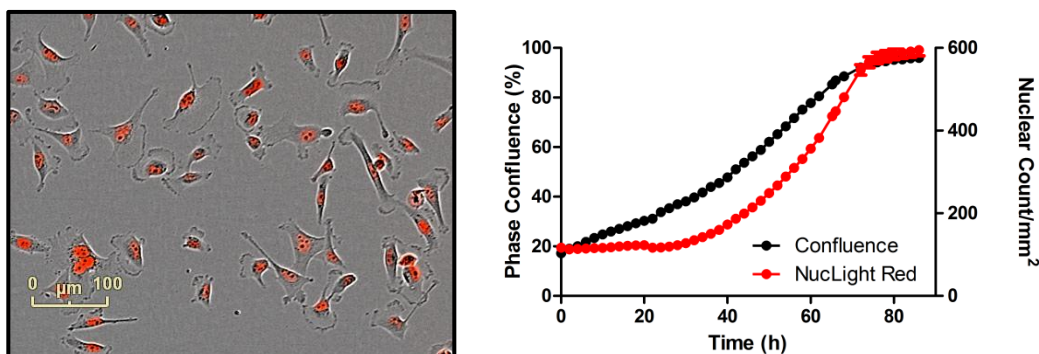


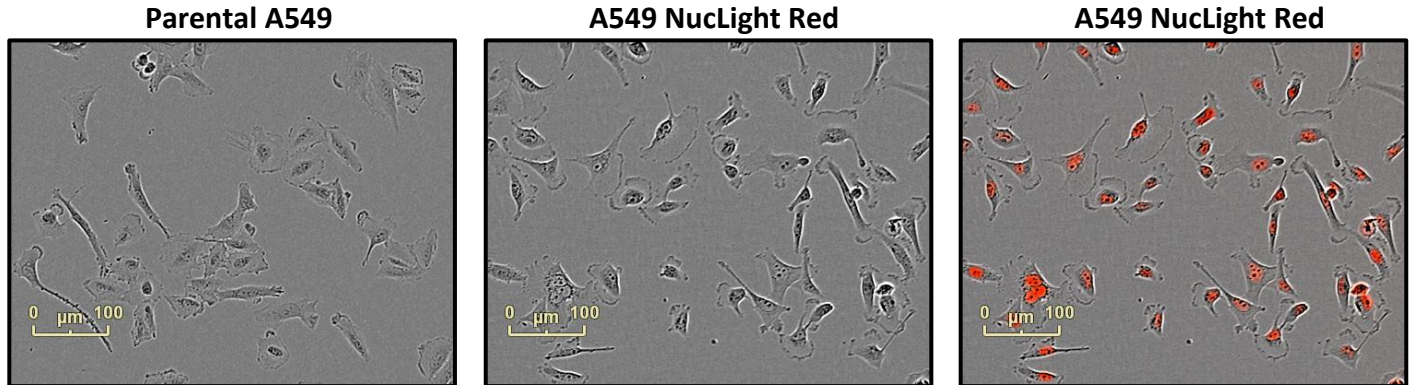
Figure 1: A549 NuLight Red Cell Line. 1 vial of A549 NuLight Red cells was thawed into a 75cm² tissue culture treated flask and imaged in IncuCyte ZOOM. Left: HD-Phase Contrast and Red Fluorescent blend. Right: Phase confluence and nuclear counts over time. A549 NuLight Red cells reach 80% confluence in a 75cm² flask after 3-4 days.



Validation Assays

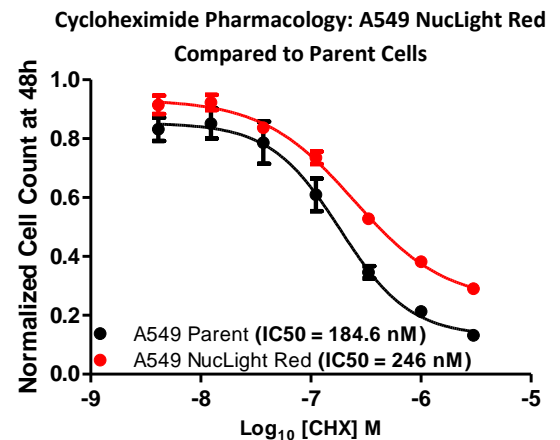
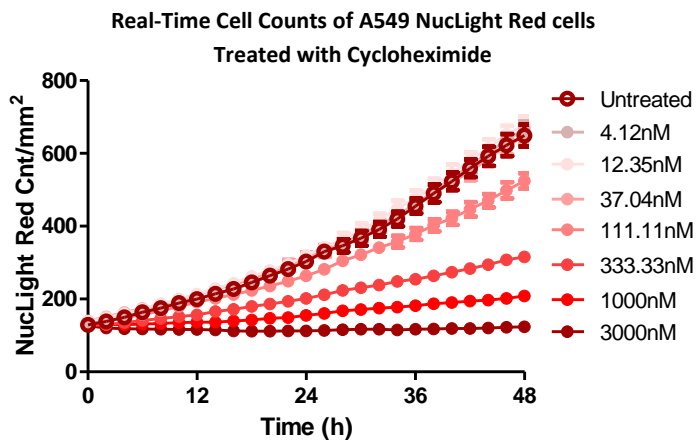
The following experiments were completed using an IncuCyte ZOOM (10x)

1. Morphological Comparison

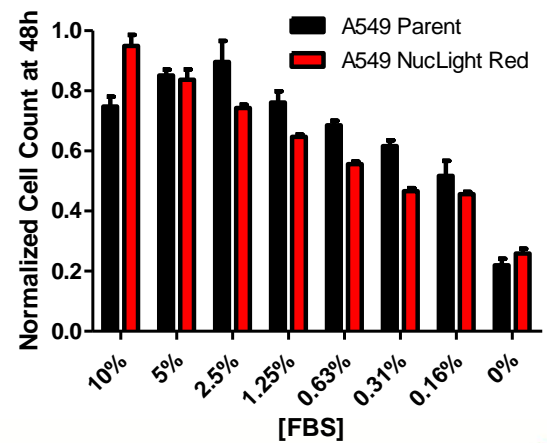


Results: No significant alterations in cell morphology were observed between parental A549 cells and the A549 NuLight Red stable population.

2. Proliferation – Real-Time Nuclear Counts



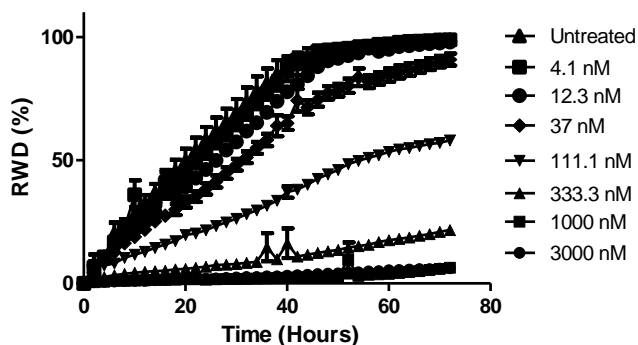
Results: Real-time cell counting revealed inhibition of A549 NuLight Red cell growth at cytostatic CHX concentrations ≥ 111 nM (Top Left). At the 48 hour endpoint, identically treated parental controls were stained with Vybrant DyeCycle Green and counted. Pharmacological analysis at the endpoint revealed similar CHX IC₅₀ concentrations for both parent and NuLight populations (Top Right). Both Parent and NuLight populations were also grown in decreasing serum conditions. Endpoint values revealed similar growth characteristics in all concentrations (Right).



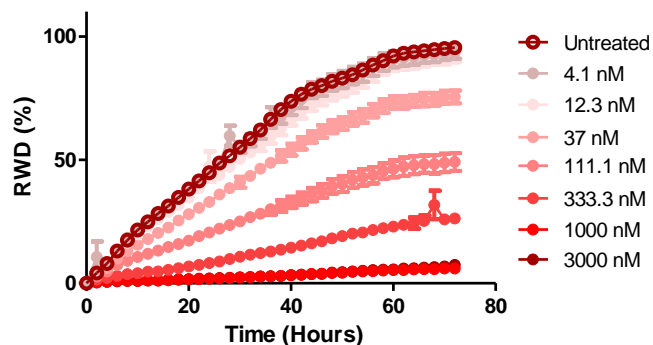


3. Cell Migration

Kinetic Pharmacology: Cytochalasin D Treated Parental A549 Cells

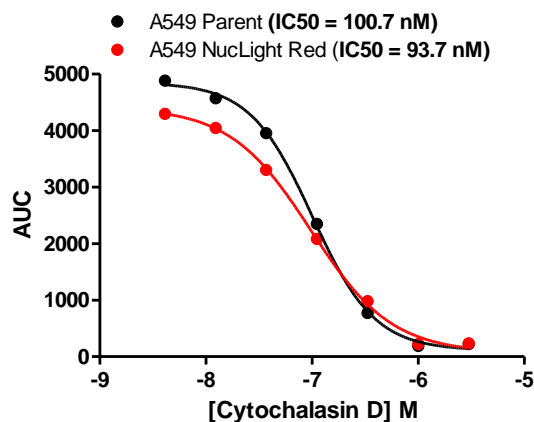


Kinetic Pharmacology: Cytochalasin D treated A549 NuLight Red cells



Results: The migration kinetics of parental A549s and the stable A549 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 was observed in both parental and stable A549 NuLight Red cells at concentrations of CytoD ≥ 37 nM using the relative wound density (RWD) metric. Pharmacological analysis using the area under the curve (AUC) of the kinetic traces revealed similar IC50 values for cytochalasin D treatment.

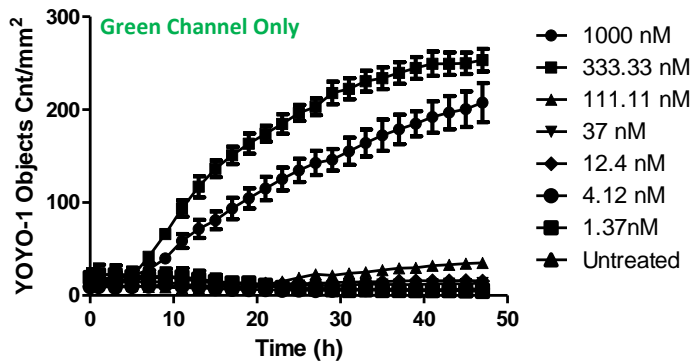
Kinetic Pharmacology: AUC Analysis and IC50 Calculation



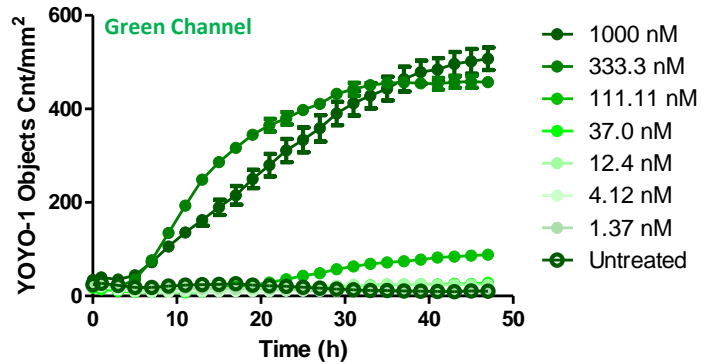


4. Cytotoxicity

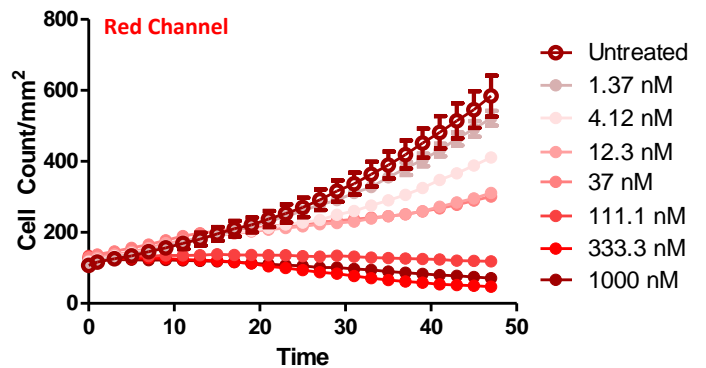
Kinetic Pharmacology: Staurosporine Treated Parental A549 Cells



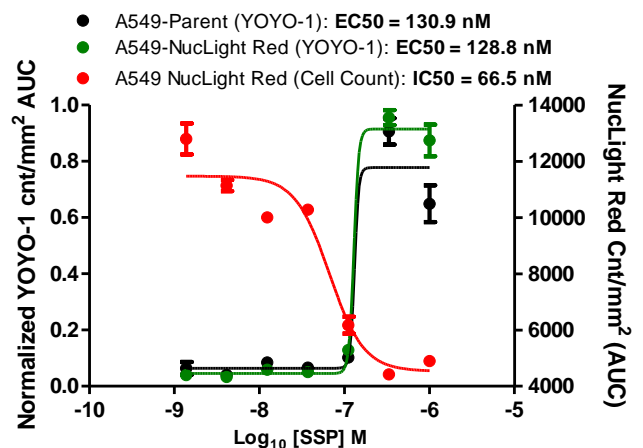
2-Color Kinetic Pharmacology: Staurosporine Treated A549 NuLight Red cells



Results: The kinetics of staurosporine (SSP) induced cell death was analyzed using the Essen 96-well Cytotoxicity protocol. YOYO-1, a cell impermeable DNA dye in the green channel, is used as a marker of cell death when it stains the DNA of the cell following loss of membrane integrity. Both the parental population and the A549 NuLight Red population exhibited similar cell death kinetics following SSP addition with a slight shift in EC50 values. The nuclear restricted marker in A549 NuLight Red cells was additionally used to determine the effect of SSP treatment on cell proliferation. Pharmacological analyses utilizing area under the curve (AUC) revealed an IC50 of approximately 66.5 nM on A549 NuLight Red proliferation.



2-Color Kinetic Pharmacology: AUC Analysis, EC50 and IC50 Calculations





Protocols and Procedures

Thawing and Culturing Cells

1. The recommended seeding density for A549 NuLight Red cells is 10,000 to 15,000 cells/cm². For example, one vial of A549 NuLight Red cells is sufficient to seed 2 to 3 T25 flasks or 1 T75 flask. From this, calculate the number of flasks needed.
2. Prepare the flasks and culture media. To maintain expression of NuLight Red label, it is recommended that cells are maintained in complete media (F-12 + 10% FBS) containing 0.5µg/ml Puromycin. Puromycin can be removed for experiment/assay set-up.
3. Remove the vial containing A549 NuLight Red from liquid nitrogen.
4. In a 37°C water bath, quickly thaw the vial by gentle agitation. Be careful not to submerge entire vial to avoid contamination. This process should take no more than 2 minutes. Remove vial when only a tiny ice crystal remains.
5. Wipe vial with 70% ethanol. In a biosafety hood, aliquot cells into sufficient complete media to distribute among the flasks set up in Step 2.
6. Gently rock the culture flasks to evenly distribute the cells and place in 37°C incubator.

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

For additional information on this and other products, please contact Essen BioScience at: sales@essenbio.com.

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