ESSEN BioSCIENCE

PRODUCT DATA SHEET

CellPlayer™ HT-1080 NucLight Red

Essen BioScience Catalog Number: 4485

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 x 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 HT-1080 cells expressing the NucLight Red fluorescent protein restricted to the nucleus. Parental HT-1080 cells were purchased from ATCC (Cat# CCL-121). HT-1080 cells were transduced with the Essen CellPlayer NucLight 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 was grown for 3-5 days in complete growth media containing 1µg/ml Puromycin to select for cells expressing NucLight Red. NucLight 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, and migration between stable populations and the parent populations from which they were derived (see below). In addition, all cells in our NucLight catalog have been certified mycoplasma free by ATCC and our stable populations have been unambiguously authenticated using ATCC's Short Tandem Repeat (STR) profiling.

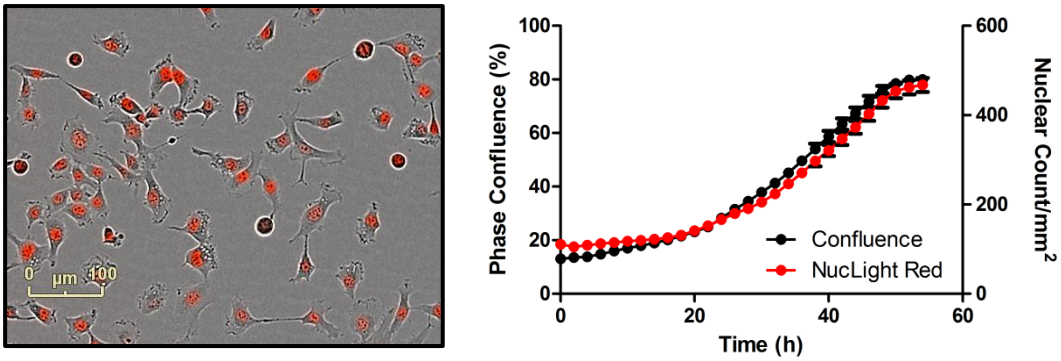


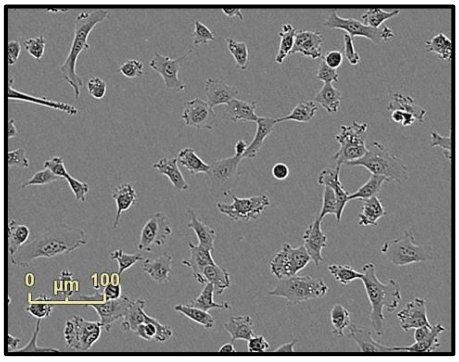
Figure 1: HT-1080 NucLight Red Cell Line. 1 vial of HT-1080 NucLight Red cells was thawed into a 75cm² tissue culture treated flask and imaged in IncuCyte ZOOM. Left: HD-Phase Contrast and Red Fluorescent blend. Right: Phase confluence and nuclear counts over time. HT-1080 NucLight Red cells reach 80% confluence in a 75cm² flask after 2-3 days.

Validation Assays

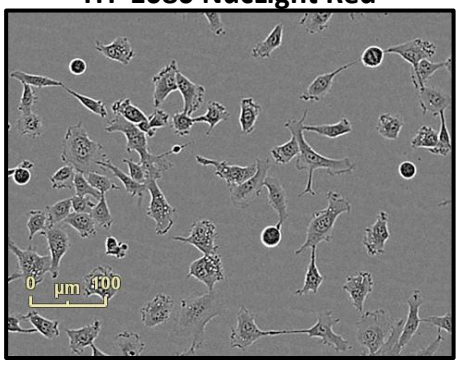
The following experiments were completed using an IncuCyte ZOOM (10x)

1. Morphological Comparison

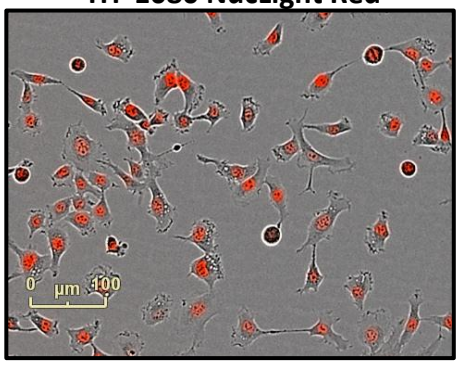
Parental HT-1080



HT-1080 NucLight Red



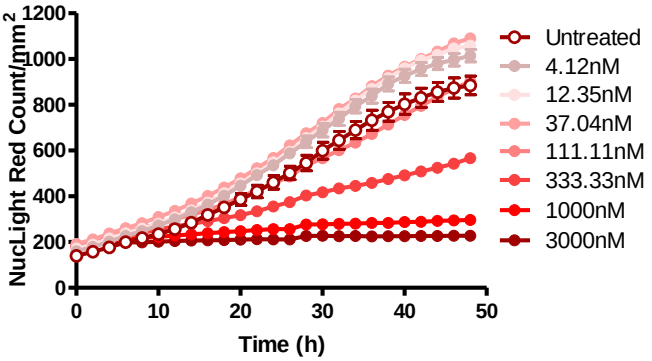
HT-1080 NucLight Red



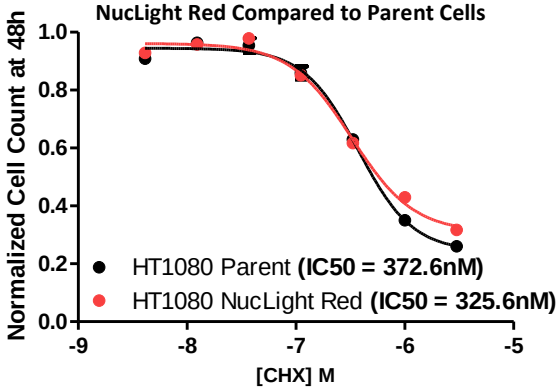
Results: No significant alterations in cell morphology were observed between parental HT-1080 cells and the HT-1080 NucLight Red stable population.

2. Proliferation – Real-Time Cell Counts Using the CellPlayer™ Kinetic Proliferation Assay

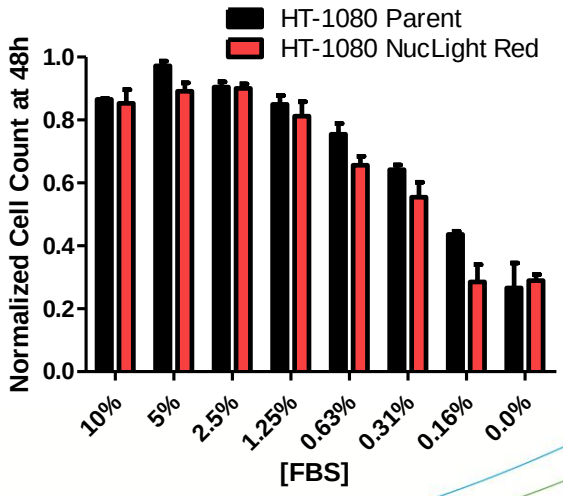
Real-Time Cell Counts of HT-1080 NucLight Red cells Treated with Cycloheximide



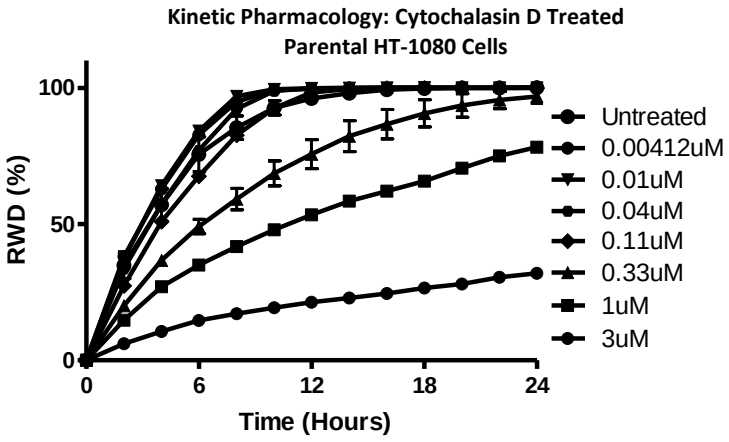
Cycloheximide Pharmacology: HT-1080 NucLight Red Compared to Parent Cells

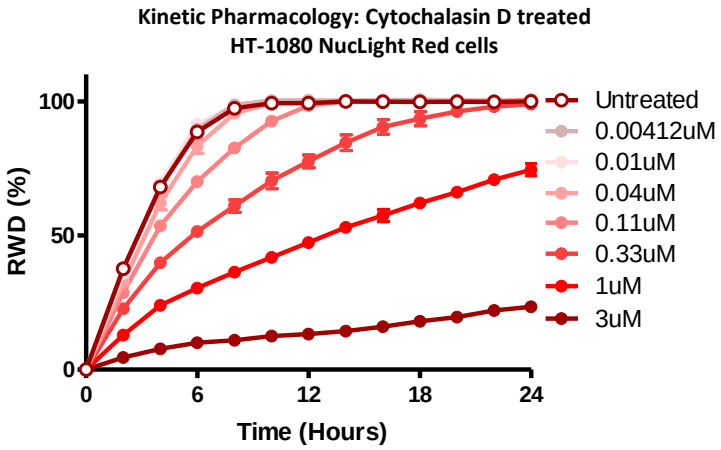


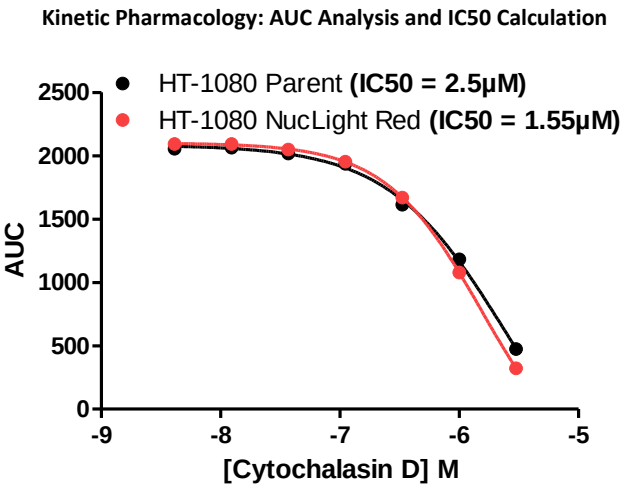
Results: Real-time cell counting revealed inhibition of HT-1080 NucLight Red cell growth at cytostatic CHX concentrations ≥333 nM (Top Left). At the 48 hour endpoint, identically treated parental controls were stained with Vybrant DyeCycle Green and counted. Pharmacological analysis at the endpoint revealed similar CHX IC50 concentrations for both parent and NucLight populations (Top Right). Both Parent and NucLight populations were also grown in decreasing serum conditions. Endpoint values revealed similar growth characteristics in all concentrations (Right).



3. Cell Migration



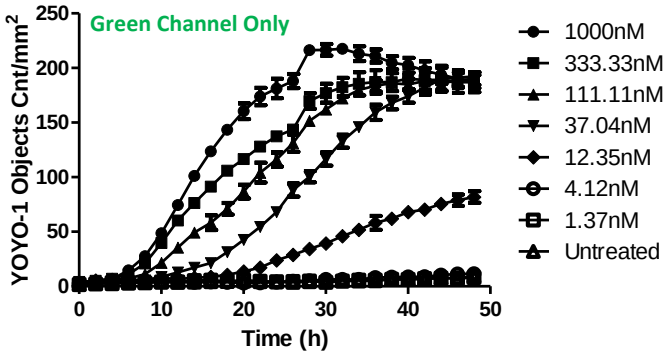




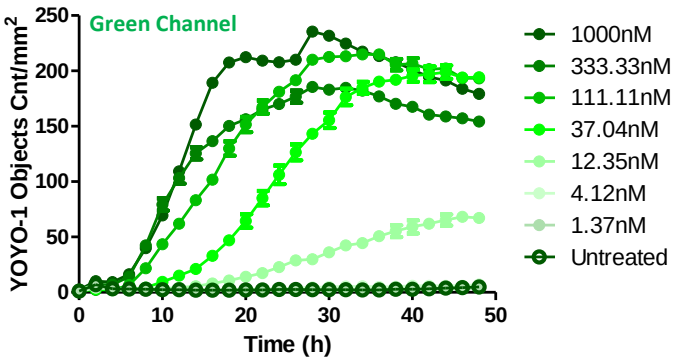
Results: The migration kinetics of parental HT-1080s and the stable HT-1080 NucLight Red population were evaluated using the label-free Essen CellPlayer 96-well Cell Migration assay in conjunction with the Essen WoundMaker tool (Cat# 4443). Cells were treated with decreasing concentrations of cytochalasin D, a potent inhibitor of actin polymerization. Concentration dependent inhibition of wound closure was observed in both parental and stable HT-1080 NucLight Red cells at concentrations of CytoD ≥0.11µM 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.

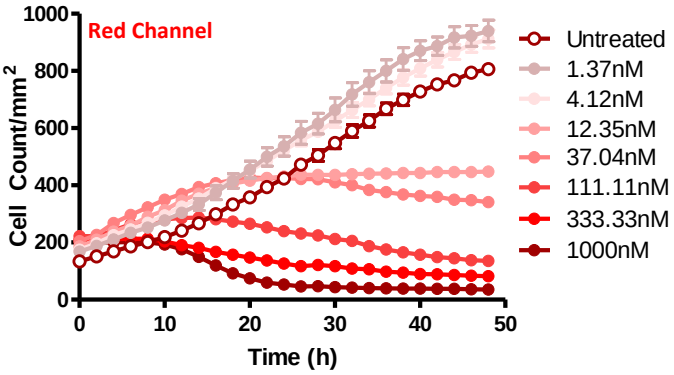
4. Cytotoxicity

Kinetic Pharmacology: Staurosporine Treated Parental HT-1080 Cells



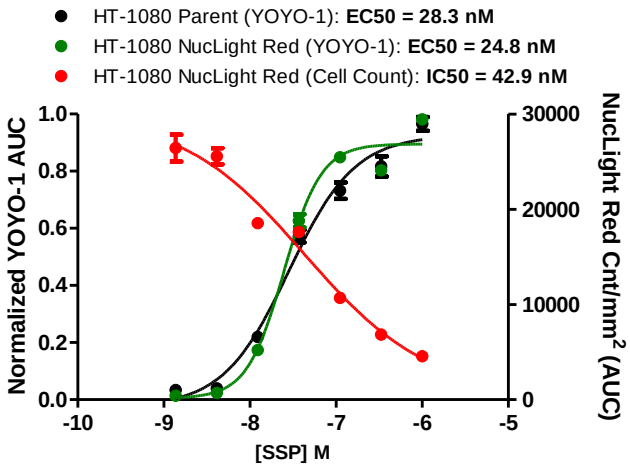
2-Color Kinetic Pharmacology: Staurosporine Treated HT-1080 NucLight Red cells





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 HT-1080 NucLight Red population exhibited similar cell death kinetics following SSP addition with nearly identical EC50 values. The nuclear restricted marker in HT-1080 NucLight 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 43 nM on HT-1080 NucLight 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 HT-1080 NucLight Red cells is 10,000 to 15,000 cells/cm^2. For example, one vial of HT-1080 NucLight Red cells is sufficient to seed 2 to 3 T25 flasks or 1 T75 flask. From this, calculate the number of flasks needed. See Figure 1 for expected growth of H-T1080 NucLight Red cells.

2. Prepare the flasks and culture media. To maintain expression of NucLight 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 HT-1080 NucLight 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

NucLight/CytoLight Reagents:

Cat.# 4475 CellPlayer NucLight Green (Lenti, EF-1 alpha, puro)
Cat.# 4476 CellPlayer NucLight Red (Lenti, EF-1 alpha, puro)
Cat.# 4481 CellPlayer CytoLight Green (Lenti, EF-1 alpha, puro)
Cat.# 4482 CellPlayer CytoLight Red (Lenti, EF-1 alpha, puro)
Cat.# 4513 CellPlayer CytoLight Green (Lenti, CMV, no selection)

NucLight Cell Lines:

Cat.# 4485 CellPlayer HT-1080 NucLight Red
Cat.# 4486 CellPlayer HT-1080 NucLight Green
Cat.# 4487 CellPlayer MDA-MB-231 NucLight Red
Cat.# 4488 CellPlayer MDA-MB-231 NucLight Green
Cat.# 4489 CellPlayer HeLa NucLight Red
Cat.# 4490 CellPlayer HeLa NucLight Green
Cat.# 4491 CellPlayer A549 NucLight Red
Cat.# 4492 CellPlayer A549 NucLight Green
Cat.# 4506 CellPlayer HUVEC NucLight Green
Cat.# 4511 CellPlayer Neuro-2a NucLight Green
Cat.# 4453 CellPlayer HUVEC CytoLight Green
Cat.# 4512 CellPlayer Neuro-2a NucLight Red

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