ESSEN BioSCIENCE

PRODUCT DATA SHEET

CellPlayer™ MDA-MB-231 NucLight Green

Essen BioScience Catalog Number: 4488

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 MDA-MB-231 cells expressing the NucLight Green fluorescent protein restricted to the nucleus. Parental MDA-MB-231 cells were purchased from ATCC (Cat# HTB-26). MDA-MB-231 cells were transduced with the Essen CellPlayer NucLight Green Lentivirus (Cat# 4475; 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 NucLight Green. NucLight Green 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 Green catalog has been certified mycoplasma free by ATCC and our stable populations have been unambiguously authenticated using ATCC's Short Tandem Repeat (STR) profiling.

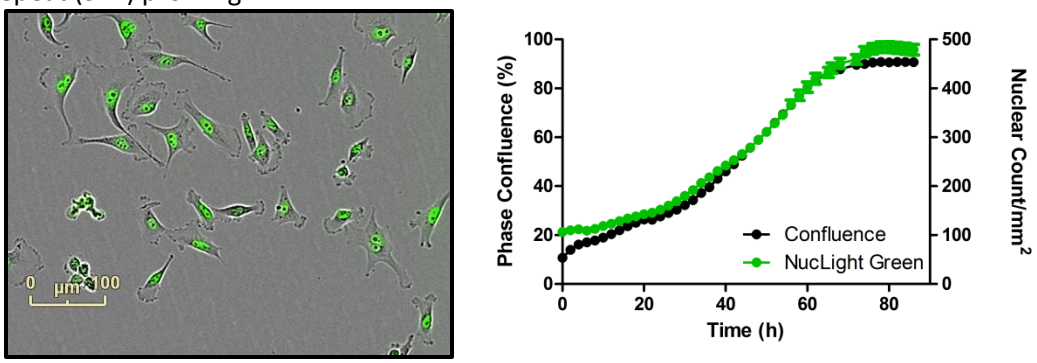


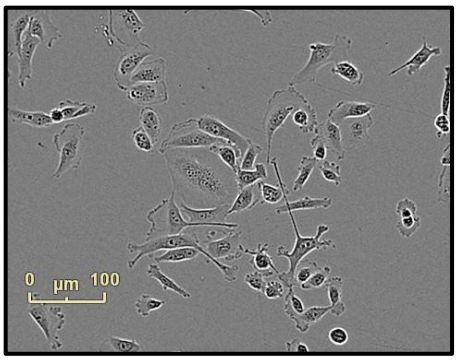
Figure 1: MDA-MB-231 NucLight Green Cell Line. 1 vial of MDA-MB-231 NucLight Green cells was thawed into a 75cm² tissue culture treated flask and imaged in IncuCyte ZOOM. Left: HD-phase contrast and green fluorescent blend. Right: Phase confluence and nuclear counts over time. Cells reach 80% confluence in a 75cm² flask after 3-4 days.

Validation Assays

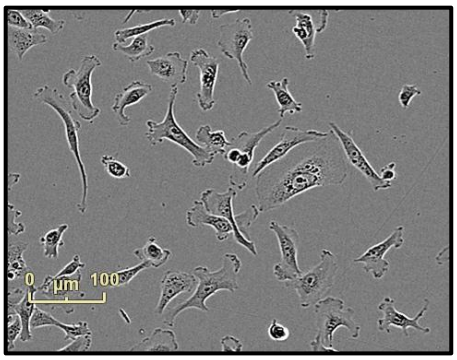
The following experiments were completed using an IncuCyte ZOOM (10x)

1. Morphological Comparison

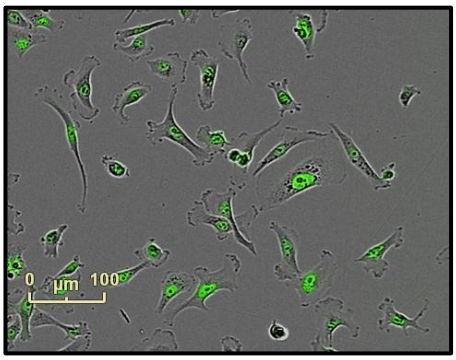
Parental MDA-MB-231



MDA-MB-231 NucLight Green



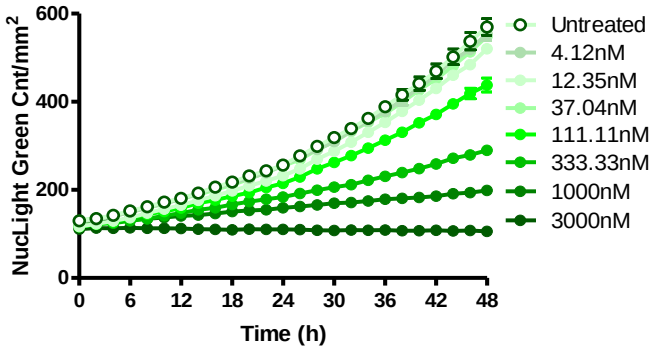
MDA-MB-231 NucLight Green



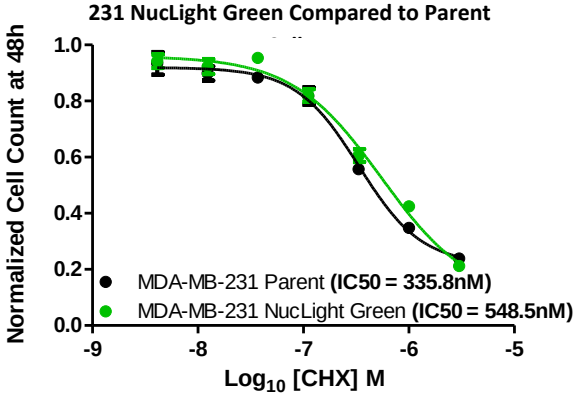
Results: No significant alterations in cell morphology were observed between parental MDA-MB-231 cells and the MDA-MB-231 NucLight Green stable population.

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

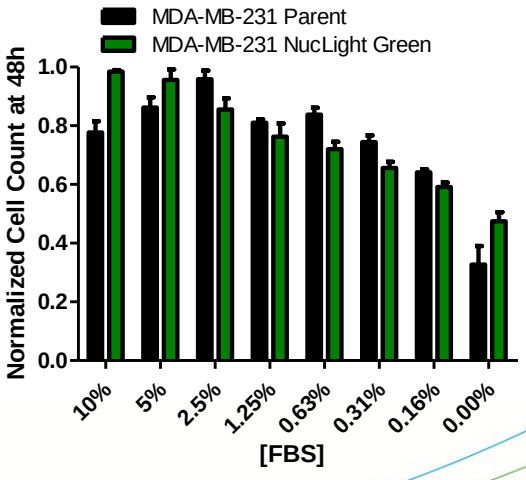
Real-Time Cell Counts of MDA-MB-231 NucLight Green cells Treated with Cycloheximide



Cycloheximide Pharmacology: MDA-MB-231 NucLight Green Compared to Parent

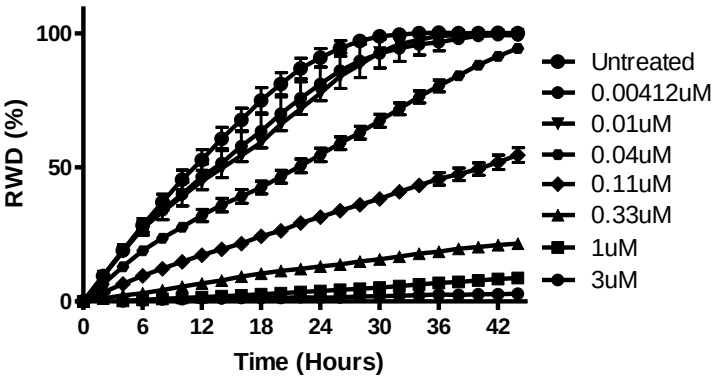


Results: Real-time cell counting revealed inhibition of MDA-MB-231 NucLight Green cell growth at cytostatic CHX concentrations ≥333 nM (Top Left). At the 48 hour endpoint, identically treated parental controls were stained with Vybrant DyeCycle Green and counted. Pharmacological analysis at the endpoint revealed slightly shifted CHX IC50 concentrations for the parent versus the 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).

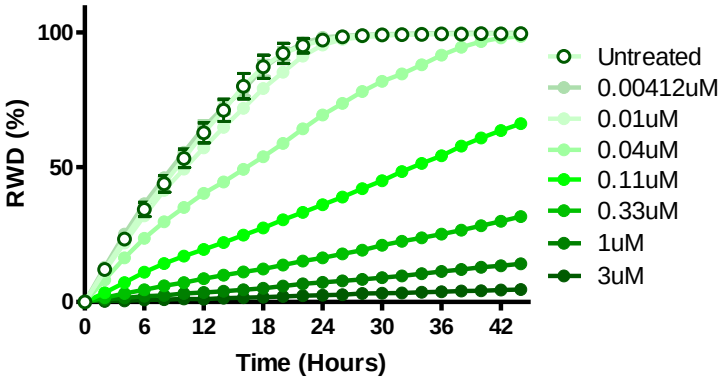


3. Cell Migration

Kinetic Pharmacology: Cytochalasin D Treated Parental MDA-MB-231 Cells

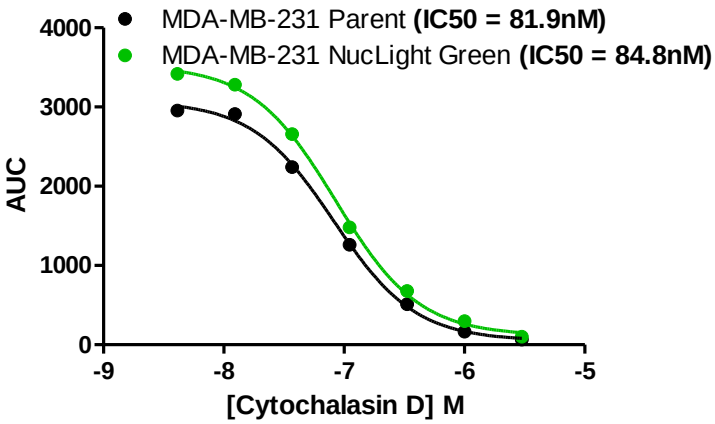


Kinetic Pharmacology: Cytochalasin D treated MDA-MB-231 NucLight Green cells



Results: The migration kinetics of parental MDA-MB-231 and the stable MDA-MB-231 NucLight Green 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 NucLight Green MDA-MB-231 cell lines at concentrations of CytoD ≥0.04µM 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



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

Protocols and Procedures

Thawing and Culturing Cells

1. The recommended seeding density for MDA-MB-231 NucLight Green cells is 10,000 to 15,000 cells/cm^2. For example, one vial of MDA-MB-231 NucLight Green 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 NucLight Green 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 MDA-MB-231 NucLight Green 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.

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

For research use only. Not for therapeutic or diagnostic use.

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").

The purchase of Essen BioScience products incorporating these fluorescent proteins conveys to the buyer the non-transferable right to use Evrogen Fluorescent Proteins only for research conducted by the buyer. No rights are conveyed to modify or clone the gene encoding fluorescent protein contained in this product or to use Evrogen Fluorescent Proteins for commercial purposes. The right to use Evrogen Fluorescent Proteins specifically excludes the right to validate or screen compounds for commercial purposes. For information on commercial licensing, contact Evrogen Licensing Department, email: license@evrogen.com.