IncuCyte® MCF7 NucLight™ Red

Catalog Number: 4524

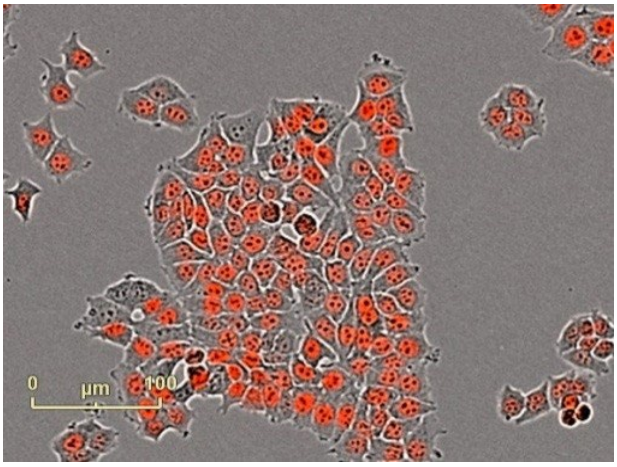
Storage
Liquid Nitrogen
Note: *Cells can be thawed and cultured immediately upon receipt or stored in liquid nitrogen for long-term storage. Storage at -80°C is not recommended. Cells should be used within 1 year of delivery.*

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

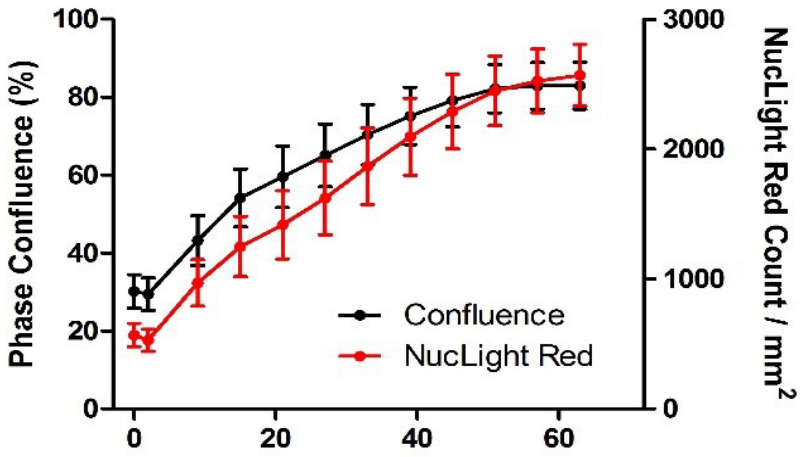
Recommended Media and Components
- Eagle's Minimum Essential Medium (EMEM) (Cat# 30-2003 ATCC)
- 10% FBS (Cat# SH30071 Thermo Hyclone)
- 0.01mg/ml Human Recombinant Insulin (Cat# #12585014 Life Technologies)
- 1% Pen/Strep (Cat# 15140 Gibco/Life Technologies)
- 0.5µg/ml Puromycin (Cat# A11138-03 Gibco/Life Technologies)

Background
Each vial contains a stable population of 1,000,000 MCF7 cells expressing the NucLight Red fluorescent protein restricted to the nucleus. Parental MCF7 cells were purchased from ATCC (Cat# HTB-22). MCF7 cells were transduced with the Essen NucLight Red 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 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 and growth/proliferation 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. MCF7 NucLight Red Cell Line
One vial of MCF7 NucLight Red cells was thawed into a 75cm² tissue culture treated flask and imaged in IncuCyte® ZOOM .



HD-phase contrast and red fluorescence blend.



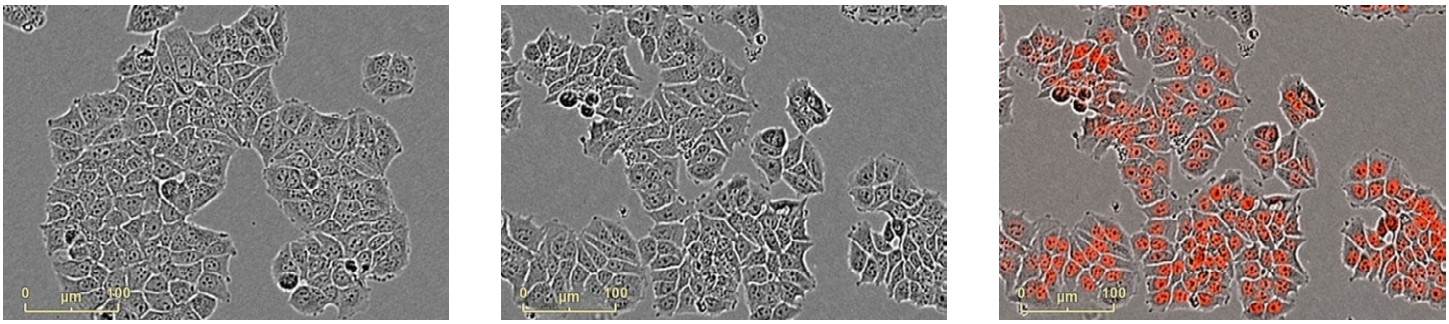
Phase confluence and nuclear counts over time.
MCF7 NucLight Red cells reach 80% confluence in a 75cm² flask after 2-3 days.

Validation Assays

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

1. Morphological comparison

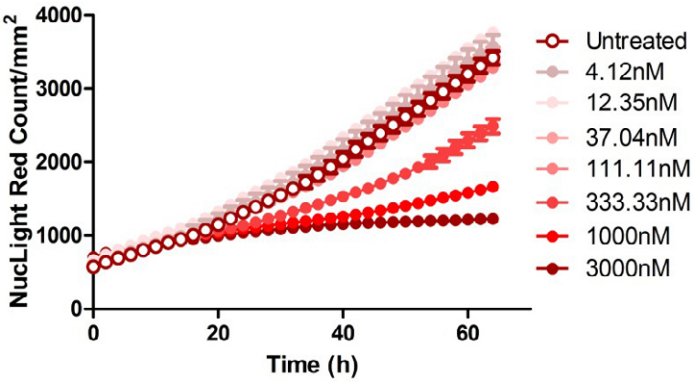
Parental MCF7 | MCF7 NucLight Red | MCF7 NucLight Red



No significant alterations in cell morphology were observed between parental MCF7 cells and the MCF7 NucLight Red stable population.

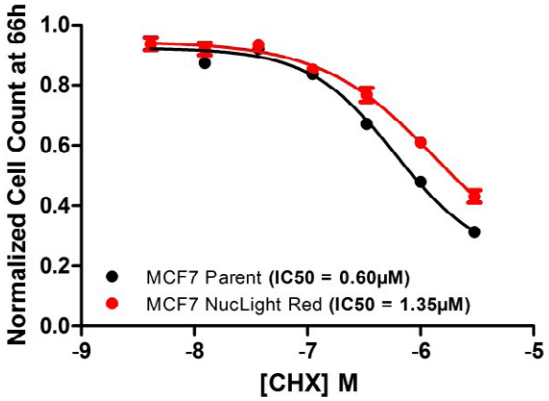
2. Proliferation – Real-time cell counts using the IncuCyte® Kinetic Proliferation Assay

Real-time cell counts of MCF7 NucLight Red cells treated with cycloheximide

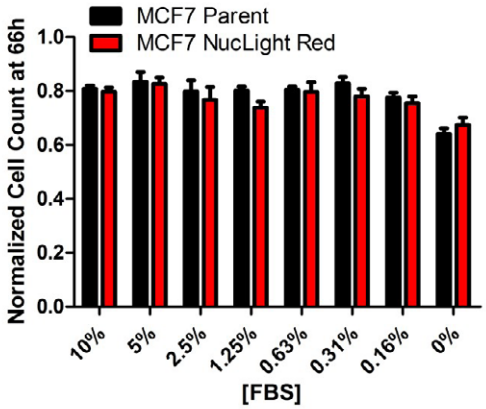


Real-time cell counting revealed inhibition of MCF7 NucLight Red cell growth at cytostatic CHX concentrations ≥333 nM.

Cycloheximide Pharmacology: MCF7 NucLight Red compared to parent cells



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



Both Parent and NucLight populations were also grown in decreasing serum conditions. Endpoint values revealed similar growth characteristics in all concentrations.

Protocol:

Thawing and Culturing Cells

1. The recommended seeding density for MCF7 NucLight Red cells is 1,500 to 2,000 cells/cm². From this, calculate the number of flasks needed.

2. Prepare the flasks and culture media. To maintain expression of NucLight Red label, it is recommended that cells are maintained in complete media (EMEM + 0.01mg/ml Bovine Insulin + 10% FBS) containing 0.5µg/ml Puromycin. Puromycin can be removed for experiment/assay set-up.

3. Remove the vial containing MCF7 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.# 4491 CellPlayer A549 NucLight Red
Cat.# 4486 CellPlayer HT-1080 NucLight Green
Cat.# 4492 CellPlayer A549 NucLight Green
Cat.# 4487 CellPlayer MDA-MB-231 NucLight Red
Cat.# 4506 CellPlayer HUVEC NucLight Green
Cat.# 4488 CellPlayer MDA-MB-231 NucLight Green
Cat.# 4511 CellPlayer Neuro-2a NucLight Green
Cat.# 4489 CellPlayer HeLa NucLight Red
Cat.# 4453 CellPlayer HUVEC CytoLight Green
Cat.# 4490 CellPlayer HeLa NucLight Green
Cat.# 4512 CellPlayer Neuro-2a NucLight Red

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