

# IncuCyte<sup>®</sup> MCF7 NuLight<sup>™</sup> Green

Catalog Number: 4528

## 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<sup>6</sup> 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)

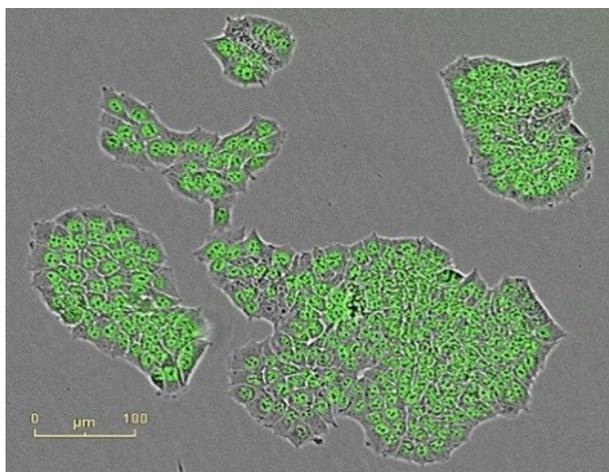
## Background

Each vial contains a stable population of 1,000,000 MCF7 cells expressing the NuLight Green fluorescent protein restricted to the nucleus. Parental MCF7 cells were purchased from ATCC (Cat# HTB-22). MCF7 cells were transduced with the Essen NuLight 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 was grown for 3-5 days in complete growth media containing 1µg/ml Puromycin to select for cells expressing NuLight Green.

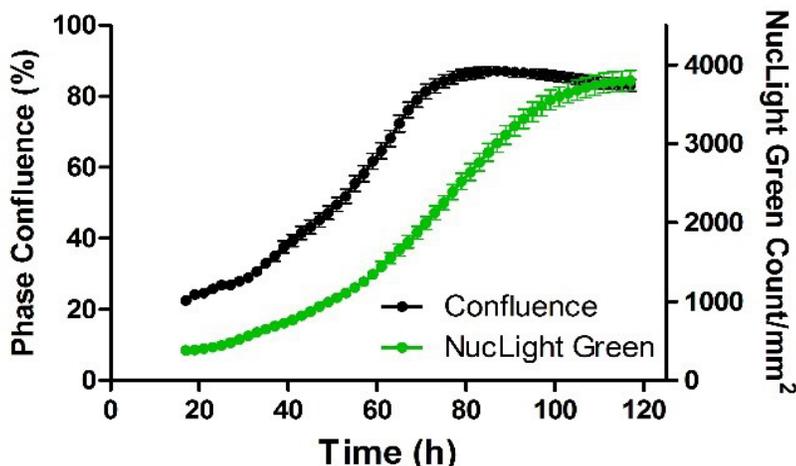
NuLight Green expressing cells are maintained in complete media containing 0.5µg/ml Puromycin. Following selection, a panel of validation assays designed to evaluate the effects of nuclear label expression on functional cell biology was completed. These assays include comparisons of cell morphology and growth/proliferation between stable populations and the parent populations from which they were derived (see below). In addition, all cells in our NuLight 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 NuLight Green Cell Line

One vial of MCF7 NuLight Green cells was thawed into a 75cm<sup>2</sup> tissue culture treated flask and imaged in IncuCyte<sup>®</sup> ZOOM .



HD-phase and green fluorescence blend.



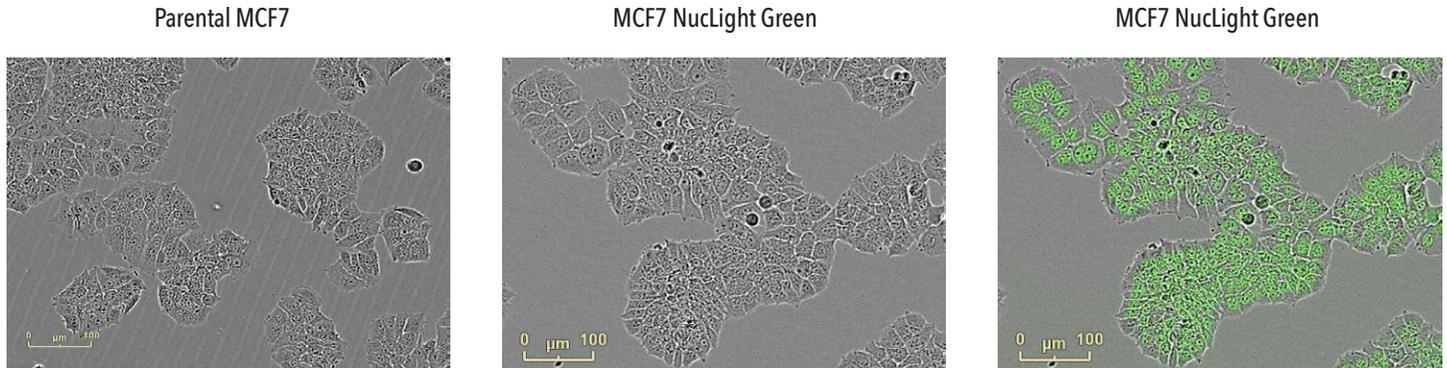
Phase confluence and nuclear counts over time.

MCF7 NuLight Green cells reach 80% confluence in a 75 cm<sup>2</sup> flask after 3-4 days.

**Validation Assays**

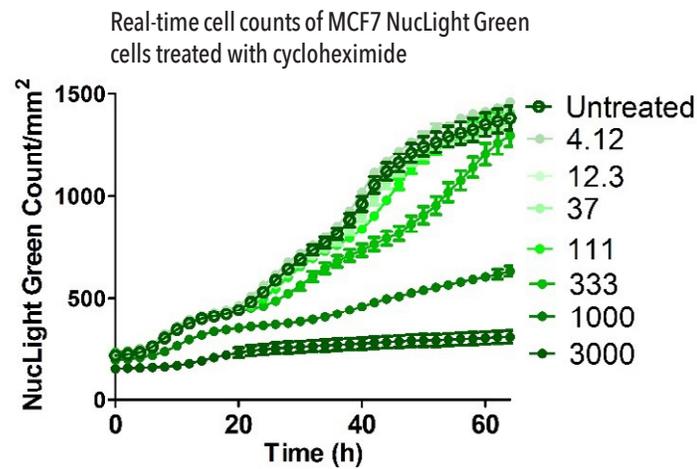
The following experiments were completed using an IncuCyte® ZOOM (10x)

**1. Morphological comparison**

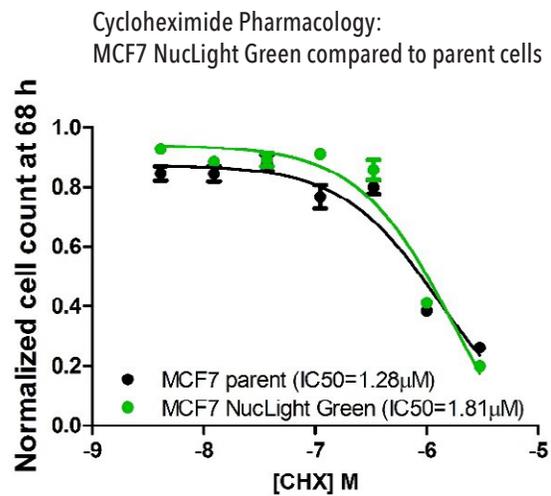


No significant alterations in cell morphology were observed between parental MCF7 cells and the MCF7 NuLight Green stable population.

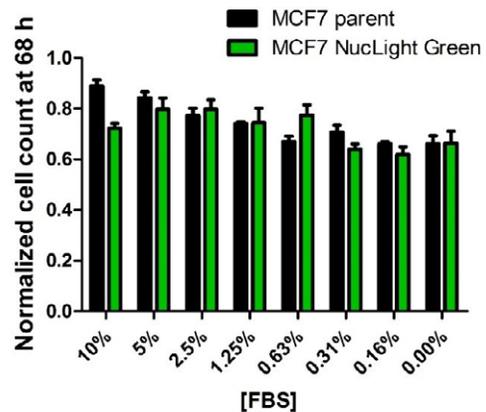
**2. Proliferation – Real-time cell counts using the IncuCyte® Kinetic Proliferation Assay**



Real-time cell counting revealed inhibition of MCF7 NuLight Green cell growth at cytostatic CHX concentrations  $\geq 333$  nM.



At the 68 hour endpoint, identically treated parental controls were stained with Nuclear-ID™ Red and counted. Pharmacological analysis at the endpoint revealed similar CHX IC50 concentrations for both parent and NuLight populations.



Both Parent and NuLight 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 NuLight Green cells is 10,000 to 15,000 cells/cm<sup>2</sup>. For example, one vial of MCF7 NuLight Green cells is sufficient to seed 2 to 3 T25 flasks or one T75 flask. From this, calculate the number of flasks needed.
2. Prepare the flasks and culture media. To maintain expression of NuLight Green 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 NuLight 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.

## 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.# 4486 CellPlayer HT-1080 NuLight Green

Cat.# 4487 CellPlayer MDA-MB-231 NuLight Red

Cat.# 4488 CellPlayer MDA-MB-231 NuLight Green

Cat.# 4489 CellPlayer HeLa NuLight Red

Cat.# 4490 CellPlayer HeLa NuLight Green

Cat.# 4491 CellPlayer A549 NuLight Red

Cat.# 4492 CellPlayer A549 NuLight Green

Cat.# 4506 CellPlayer HUVEC NuLight Green

Cat.# 4511 CellPlayer Neuro-2a NuLight Green

Cat.# 4453 CellPlayer HUVEC CytoLight Green

Cat.# 4512 CellPlayer Neuro-2a NuLight Red

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