ESSEN BioSCIENCE

PRODUCT DATA SHEET

CellPlayer™ NucLight Mix:

HT-1080 NucLight Green and A549 NucLight Red

Essen BioScience Catalog Number: 4516

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

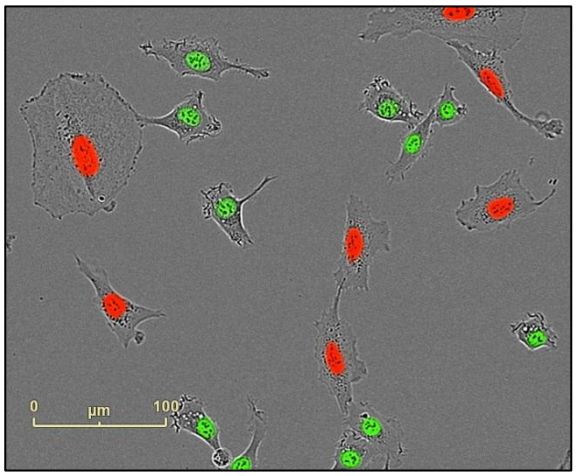
- HT-1080 NucLight Green: 5x10^5 cells/vial
- A549 NucLight Red: 5x10^5 cells/vial

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 mixed stable population of 500,000 HT-1080 NucLight Green cells (Cat# 4486) and 500,000 A549 NucLight Red cells (Cat# 4491). Parental HT-1080 and A549 cells were purchased from ATCC (Cat# CCL-121 and Cat# CCL-185, respectively). HT-1080 and A549 cells were made individually using the Essen CellPlayer NucLight Green Lentivirus (Cat# 4475; EF1α, puromycin) and NucLight Red Lentivirus (Cat# 4476; EF1α, puromycin), respectively, 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. Individual cell lines were mixed 1:1 to a total final concentration of 1 million cells/mL in 90% FBS and 10% DMSO. All individual cell lines in our catalog have been certified mycoplasma free by ATCC and our stable populations have been unambiguously authenticated using ATCC's Short Tandem Repeat (STR) profiling.



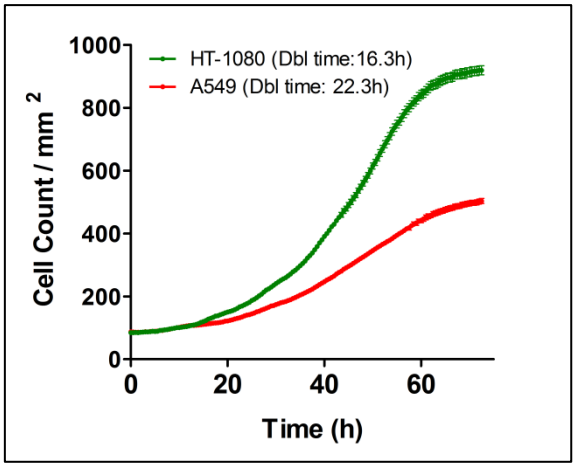


Figure 1. (Top) HD-Phase and fluorescence blend of HT-1080 NucLight Green and A549 NucLight Red cells imaged at 20x in IncuCyte ZOOM. (Bottom) Proliferation and doubling times of each cell type in co-culture (4x).

Protocols and Procedures

Thawing and Culturing Cells

1. Immediately thaw vial of NucLight Mix cells, or store in liquid nitrogen upon receipt.

2. In a 37°C water bath, quickly thaw each 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.

3. Wipe vial with 70% ethanol and perform step 4 or 5 in a biosafety hood.

4. Prepare a T75 Flask
    a. Dispense 5mL culture media into a T25 flask.
    b. Aliquot cells into the T25 flask.
    c. Gently rock the culture flasks to evenly distribute the cells and place in 37°C incubator.
    d. 5-8hr later, replace media containing DMSO with fresh media.

5. Prepare a Microplate
    a. Dispense 5-10mL media into a 15mL conical tube
    b. Aliquot cells into the 15mL conical tube and centrifuge at 1,000rpm for 5 minutes.
    c. Aspirate media, re-suspend the cell pellet in fresh media, and distribute to wells according to Table 1.
    d. Let cells sit at room temperature for a minimum time of 30 minutes before incubating at 37°C.

Table 1: Recommended volumes and final cell densities for microplates

Microplate Vessel	Volume to re-suspend cell pellet	Volume to add per well	Final cell density per well
6-well	15mL	2.3mL	~150,000 cells/well
12-well	13mL	1mL	~75,000 cells/well
24-well	13mL	0.5mL	~35,000 cells/well
48-well	13mL	0.25mL	~20,000 cells/well
96-well	12mL	0.1mL	~8,000 cells/well

IncuCyte ZOOM™ Set-up and Data Analysis

1. Immediately place the flask or microplate in IncuCyte ZOOM™. Let the flask or plate warm up for 15-30 minutes.

2. Set the Scan Schedule:
    a. Channel Selection: Phase, Green, Red
    b. Spectral Unmixing: 8% of Red removed from Green
    c. Set interval every 2 hours
    d. Job Type: Basic Analyzer
    e. Processing Definition: Phase/Green/Red Processor (4x), (10x), or (20x)

3. Scan for 3-5 days, collect 4-6 images representative of the mixed culture
    a. Create a processing definition and launch an analysis job following the Fluorescence Processing Technical Note.
    b. Analyze data following the Proliferation Application Note.

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

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

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