ESSEN BioSCIENCE

PRODUCT DATA SHEET

CellPlayer™ Neuro-2a NucLight Red

Essen BioScience Catalog Number: 4512

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, 2 x 10^6 cells/mL in 95% growth medium (without puromycin), 5% DMSO

Recommended Media and Components

F-12K (Cat# 30-2004 ATCC)
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 2,000,000 Neuro-2a cells expressing the NucLight Red fluorescent protein, restricted to the nucleus. Parent Neuro-2a cells were purchased from ATCC (Cat# CCL-131). Neuro-2a cells were transduced with the Essen CellPlayer NucLight Red Lentivirus (Cat# 4476; EF1α, puromycin). 48 hours post infection, the complete population of cells were grown for 3-5 days in complete growth media containing 2 µg/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 neurite outgrowth between stable populations and the parent populations from which they were derived (see below). In addition, our complete NucLight Red cell catalog has been certified mycoplasma free by ATCC and the stable populations of Neuro-2a have been authenticated using Idexx Radil's CellCheck™ analysis.

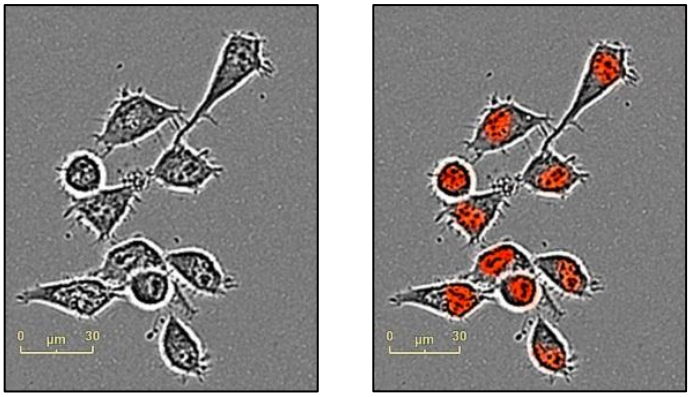


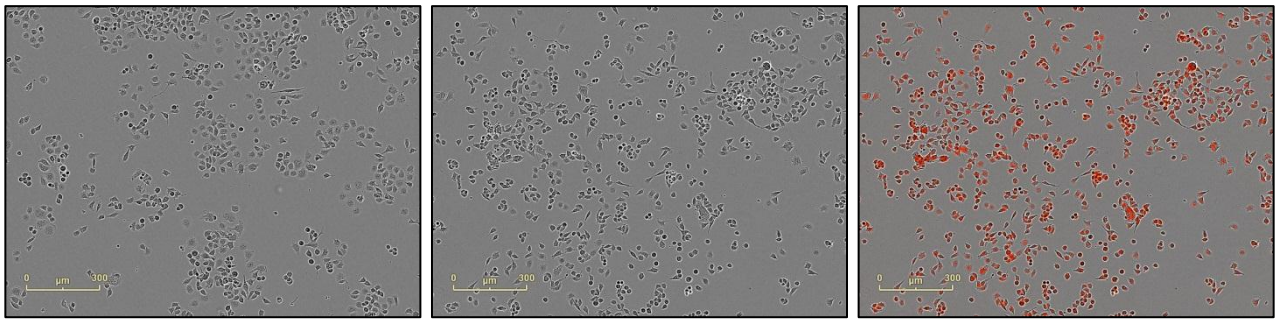
Figure 1. Neuro-2a NucLight Red Cell Line. Left: Phase Contrast; Right: HD-Phase and fluorescence blend.

Validation Assays

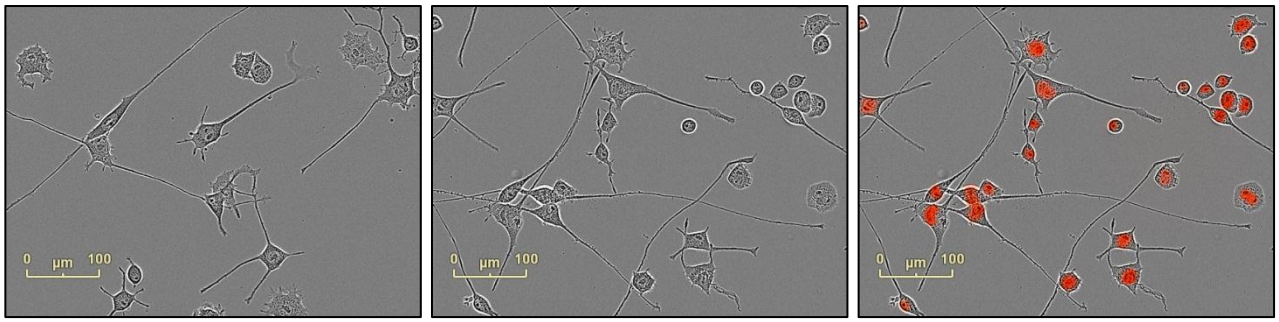
The following experiments were completed using an IncuCyte ZOOM (10x and 20x)

1. Morphological Comparison

Parental Neuro-2a Proliferating | **Neuro-2a NucLight Red Proliferating** | **Neuro-2a NucLight Red Proliferating**

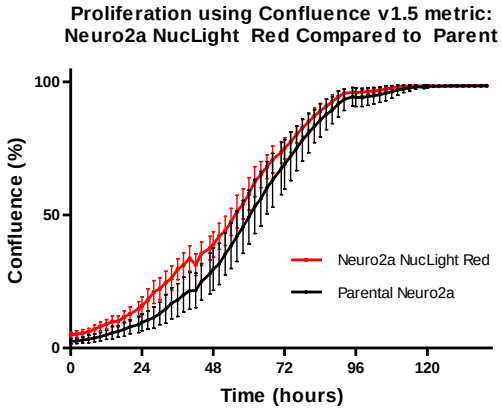


Parental Neuro-2a Differentiating | **Neuro-2a NucLight Red Differentiating** | **Neuro-2a NucLight Red Differentiating**



Results: The Neuro-2a NucLight Red population was observed to have slightly smaller average cell body area than the parent line in the proliferative state (top row, images taken in an IncuCyte ZOOM at 10x). More cells with a flatter, more adherent morphology are evident in the parent line. In the differentiated state, morphology was similar between the two lines, (bottom row, images taken in an IncuCyte ZOOM at 20x) as seen 4 days after treatment of 20 µM all-*trans* retinoic acid.

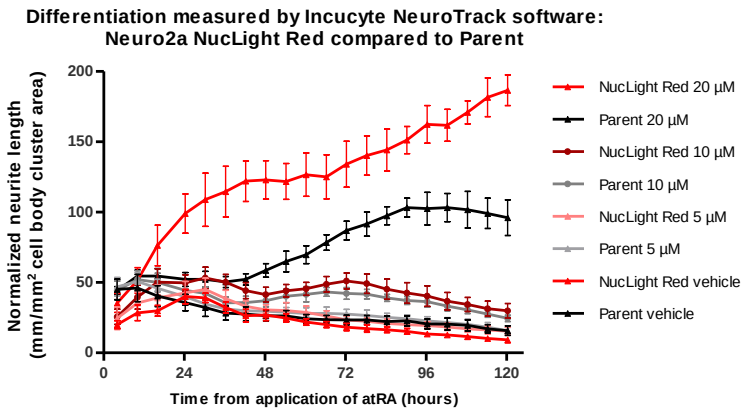
2. Proliferation –Confluence v1.5 Metric



Methods: Parent Neuro-2a and Neuro-2a NucLight Red cells were plated at a density of 2,000 cells per well in a 96-well plate in F-12K supplemented with 10% FBS (N=12). A half medium change was made after 4 days. Images were captured at 2 hour intervals in a 10x IncuCyte FLR in HD-phase. The Confluence v1.5 metric was utilized to measure proliferation. Chart was made in GraphPad Prism 5.0

Results: Over the course of 7 days, no differences in proliferation were detected when comparing parental Neuro-2a cells to the stable Neuro-2a population expressing NucLight Red.

3. Neurite Outgrowth



Methods: Parent Neuro-2a and Neuro-2a NucLight Red cells were plated at a density of 2,000 cells per well in a 96-well plate in serum-free F-12K (N=8). Four hours after plating, FBS and all-*trans* retinoic acid (atRA) were added for a final concentration of 2% serum and the indicated final concentrations of atRA. The DMSO vehicle was used at a concentration previously shown to be non-perturbing (0.09%). Images were captured at 6 hour intervals in an IncuCyte ZOOM at 20x in phase. The NeuroTrack metric was utilized to measure neurite length. NeuroTrack offers the option of normalizing neurite length to a nuclear marker, to cell body cluster count, or to cell body cluster area. Because the parental line has no nuclear marker and because cell body clusters were so large due to cell proliferation, cell body cluster area was utilized as the normalizing factor, which assured that the normalized neurite length metric accounts for cell number and represents neurite outgrowth. Charts were made in GraphPad Prism 5.0.

Results: The application of atRA produced a concentration-dependent change in neurite outgrowth in both the parent Neuro-2a and the NucLight Red populations. At 5 µM atRA, there was no effect on the neurite growth compared to the vehicle. At 10 µM atRA, the NucLight Red and the parent cells showed an initial increased neurite length per cell body cluster area. However, this effect was minimized as the normalization parameter continued to increase as a result of cell proliferation. At 20 µM atRA, both populations showed strong induction of differentiation and neurite outgrowth. The increase in neurite length demonstrated that the NucLight Red cells are at least as sensitive to atRA as the parent line.

Protocols and Procedures

Thawing and Culturing Cells

1. The recommended seeding density for Neuro-2a NucLight Red cells is 6,000 cells/cm^2. From this, calculate the number of flasks needed.

2. Prepare the plate and culture media. To maintain expression of NucLight Red label, it is recommended that cells are maintained in complete media (F-12K + 10% FBS) containing 0.5µg/ml Puromycin. Puromycin can be removed for experiment/assay set-up.

3. Remove the vial containing Neuro-2a 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 flasks to evenly distribute the cells and place in 37°C incubator at 5% CO_2. Doubling time is approximately 14 hours.

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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