Protocol

IncuCyte® Nuclight Rapid Red Cell Labeling

For the nuclear labeling of cells

This protocol provides an overview of the IncuCyte® NucLight Rapid Cell Labeling Reagent methodology. It is compatible with the IncuCyte® S3 Live-Cell Analysis System and describes how IncuCyte® NucLight Rapid Red can be used to fluorescently label the nucleus of living cells without perturbing cell function or biology. In addition, this reagent can be multiplexed with IncuCyte® Annexin V Green, Caspase-3/7 Green, or Cytotox Green reagents for simultaneous readouts of apoptosis or cytotoxicity in the same well, without perturbing cell function or biology. In addition, this reagent can be multiplexed with IncuCyte® Annexin V Green, Caspase-3/7 Green, or Cytotox Green reagents for simultaneous readouts of apoptosis or cytotoxicity in the same well.

Required materials

- IncuCyte NucLight Rapid Red Reagent (Sartorius Cat# 4717)
- 0.01% Poly-L-ornithine solution (Sigma P4957) – optional, for non-adherent cells
- Flat bottom tissue culture plate (e.g., Corning 3595) Required materials

General Guidelines

- Protect IncuCyte NucLight Rapid Red Reagent from light at all times.
- Following cell seeding, place plates at ambient temperature (30 minutes) to ensure homogenous cell settling.
- Remove bubbles from all wells by gently squeezing a wash bottle (containing 70-100% ethanol with the inner straw removed) to blow vapor over the surface of each well.
- After placing the plate in the IncuCyte® S3 Live-Cell Analysis System, allow the plate to warm to 37 °C for 30 minutes prior to scanning
- If using non-adherent cells (e.g. immune cells) we recommend coating plates with 0.01% poly-L-ornithine solution (as supplied) or 5 µg/mL fibronectin diluted in 0.1% BSA/PBS (Ca+/Mg+ - free) to prevent cell aggregation at well edges. For a 96-well plate, add 50 µL of chosen matrix solution to each well, incubate for 1 hour at ambient temperature, remove solution from wells and then allow plates to dry for 30-60 minutes prior to cell addition. Plates may be coated the day before and stored, once dried, overnight at 4°C.
- For optimal results, it is recommended to utilize the highest non-perturbing concentration of the IncuCyte NucLight Rapid Red reagent when labeling cells. To determine this concentration, perform an initial optimization experiment as described below. It is recommended to perform an optimization experiment each time a new cell type is used, as the optimal final assay concentration will vary.

Preperation of IncuCyte NucLight Rapid Red Reagent

1. Prepare Stock Concentration

1.1 Prior to harvesting cells, bring one or more vials of IncuCyte NucLight Rapid Red to room temperature and briefly centrifuge to ensure the reagent is located in the bottom of the vial.

2. Prepare nuclight rapid working concentration in complete media

2.1 For initial cell labeling optimization, dilute the stock to yield a 2x working concentration. To yield a working dilution of 1:125, add 3 µL of NucLight Rapid Reagent to 372 µL of complete media.

2.2 Perform a 2-fold serial dilution of the NucLight Rapid reagent working stock (180 µL of the 1:125 dilution to 180 µL complete media) to create 5 test concentrations in triplicates.

NOTE: The final recommended dilutions of the reagent will be 1:250, 1:500, 1:1000, 1:2000 and 1:4000 when added to the assay plate. We have found that 1:500 is a reasonable concentration for most cell type worked tested.

This reagent is supplied in (100%) DMSO, thus, for the unlabeled control cells, we recommend adding equivalent volumes of DMSO to ensure that DMSO does not perturb cell health.

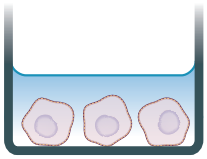
Use the phase-contrast confluence metric to monitor both cell proliferation and the red object count metric to monitor labeling efficiency.

Alternatively use the IncuCyte Cell-by-Cell Analysis Software Module (PN 9600 0031) to monitor cell count and labeling efficiency of cells masked individually.
For further details of this analysis module and it's application see:

www.essenbioscience.com/cell-by-cell

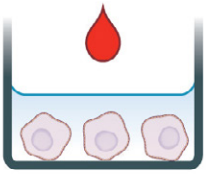
Nuclear Cell Labeling Protocol

1. Seed cells



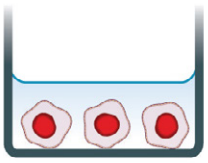
Seed cells (50 µL/well, 1,000 – 5,000) into a 96-well plate.

2. Label



Add IncuCyte® NucLight Rapid live-cell labeling reagent.

3. Acquire images



Capture images every 2–3 hours (4x, 10x or 20x) in IncuCyte® S3 Live-Cell Analysis System. Analyze using integrated software.

1. Seed cells

1.1. Harvest cells using a suitable dissociation solution, then neutralize with complete media.

1.2. Count and prepare cells at desired density (50 µL per well) for assay.

NOTE: A 50µL/well seeding volume is recommended in order to accommodate the volume of NucLight Red labeling reagent. If preparing a plate for a multiplexed functional assay, we recommend seeding cells in a 100 µL volume and incubating overnight prior to cell labeling and other reagent and treatment additions.

2. Label cells

2.1. Dilute NucLight Rapid Red Reagent in complete media at the dilution previously determined in the optimization assay.

2.2. Immediately add 50 µL of diluted NucLight Rapid Red to wells containing cells.

NOTE: If performing multiplexed functional assays, dilute the NucLight Rapid Red and IncuCyte Cell Health Reagent (e.g. Caspase-3/7, Annexin V or CytoTox Green) ± treatments in complete media to ensure a final assay concentration of 1x. Aspirate media from plate containing cells, and immediately add diluted reagents ± treatments.

2.3. Allow cells to settle at room temperature for 30 minutes before placing the plate in IncuCyte® S3 Live-Cell Analysis System.

3. Image Plate

3.1. Place the plate into the IncuCyte® S3 Live-Cell Analysis System to monitor both cell proliferation using the phase contrast confluence metric and labeling efficiency using the red fluorescence channel. If adding a green cell health reagent, onset of cell death via the reagent should also be monitored using all three channels (Phase + red fluorescence + green fluorescence).

a. Objective: 4x, 10x or 20x
b. Channel selection: Phase + Fluorescence
c. Scan type: Standard
d. Scan interval: Typically, every 2 hours

NOTE: When using IncuCyte Cell-by-Cell Analysis Software Module:

a. Scan type: Standard/Adherent Cell-by-Cell
b. Objective: 10x

Analysis guidelines

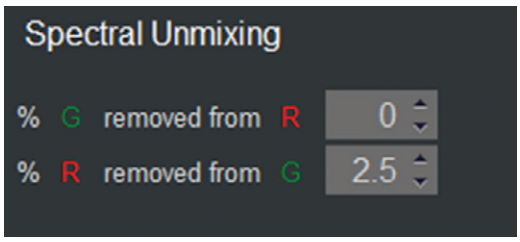
In some cell types, the IncuCyte NucLight Rapid Red reagent may label both live and dead cells. In this circumstance, unprocessed, raw images are auto-scaled to the brightest objects (typically the dead cells), giving the deceptive appearance that live-cells are not labeled. In order to correctly analyze images where both dead and live cells are stained we recommend the following:

a. When adding images for analysis, include wells that contain both dead (bright) and live (dim) cells from multiple time points (e.g. t = 0, 24, 48 hours).
b. Hover over the images to evaluate the Red Calibrated Unit (RCU) of both the bright and dim objects. Use these values to define your segmentation values for inclusion of live cells as well as to filter out dead cells based on their mean intensity values.

NOTE: When using the IncuCyte Cell-by-Cell Analysis Software Module it is possible to classify cells based on the presence/absence of fluorescence along with other shape change metrics.

Evaluating results to determine optimal reagent concentration

Use the IncuCyte system HD phase images and confluence metrics to compare the cell morphology and growth rates for each concentration of reagent and compare to the non-labelled control cells. Alternatively using the IncuCyte Cell-by-Cell Analysis Software Module you can assess individual cell count and shape changes of individual cells. The optimal concentration of IncuCyte® NucLight Rapid Reagent is the highest concentration that does not cause significant changes to growth rate or morphology while providing efficient cell labeling. If multiplexing, it is important that the spectral un-mixing values are set to "2.5% Red removed from Green", in order to reduce spectral emission crossover from the red channel to the green channel. This provides for successful masking with our green cell toxicity reagents when combined with NucLight Rapid Red reagent.



A complete suite of cell health applications is available to fit your experimental needs. Find more information at **essenbioscience.com**

For additional product or technical information,
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