SARTORIUS

Product Guide

Incucyte® Nuclight Rapid Red Dye

For Nuclear Labeling of Live Cells

Product Information

Presentation, Storage and Stability

Incucyte® Nuclight Rapid Red Dye is supplied as a single vial (50 µL) in dimethylsulfoxide (DMSO), with each vial providing sufficient quantity for performing 100–200 tests (1 test = 1 well of a 96-well microtiter plate). Upon receipt, the solution should be stored at 4° C. When stored as described, the stock solutions will be stable for at least 12 months.

Product Name	Cat. No.	Ex. Max	Em. Max	Storage	Stability
Compatible with Incucyte® Live-Cell Analysis Systems configured with Green | Red Optical Module					
Incucyte® Nuclight Rapid Red Dye	4717	655 nm	681 nm	4° C	12 months from date of receipt

Safety data sheet (SDS) information can be found on our website at **www.sartorius.com**

Background

The Incucyte® Rapid Red Dye for cell labeling is a cell permeable DNA stain that specifically stains nuclei in cells and is ideally suited to the mix-and-read, real-time quantification of cell counting. Addition of the Incucyte® Nuclight Rapid Red Dye to normal healthy cells is non-perturbing to cell growth and morphology and provides homogenous staining of nuclei. When added to tissue culture medium, the inert stain crosses the cell membrane and has excellent specificity for DNA without the need for a wash step. With the Incucyte® integrated analysis software, fluorescent objects can be quantified and background fluorescence minimized. This reagent has been validated for use with the Incucyte® Live-Cell Analysis System and enables the real-time quantification of cell proliferation. Furthermore, our Incucyte® Nuclight Rapid Red Dye can be combined with Incucyte® Cell Health Reagents to quantify cell proliferation alongside apoptosis or cytotoxicity in a single well.

Recommended Use

We recommend optimizing Incucyte® Nuclight Rapid Red Dye for each cell line tested by diluting the reagent in growth media (final dilutions of 1:250, 1:500, 1:1000, 1:2000 and 1:4000) and adding directly to cells in culture. Immediately post addition, cells are labeled with the Nuclight Rapid Red Dye. When used in an Incucyte® Live-Cell Analysis System, we recommend data collection every 2–3 hours.

Example Data

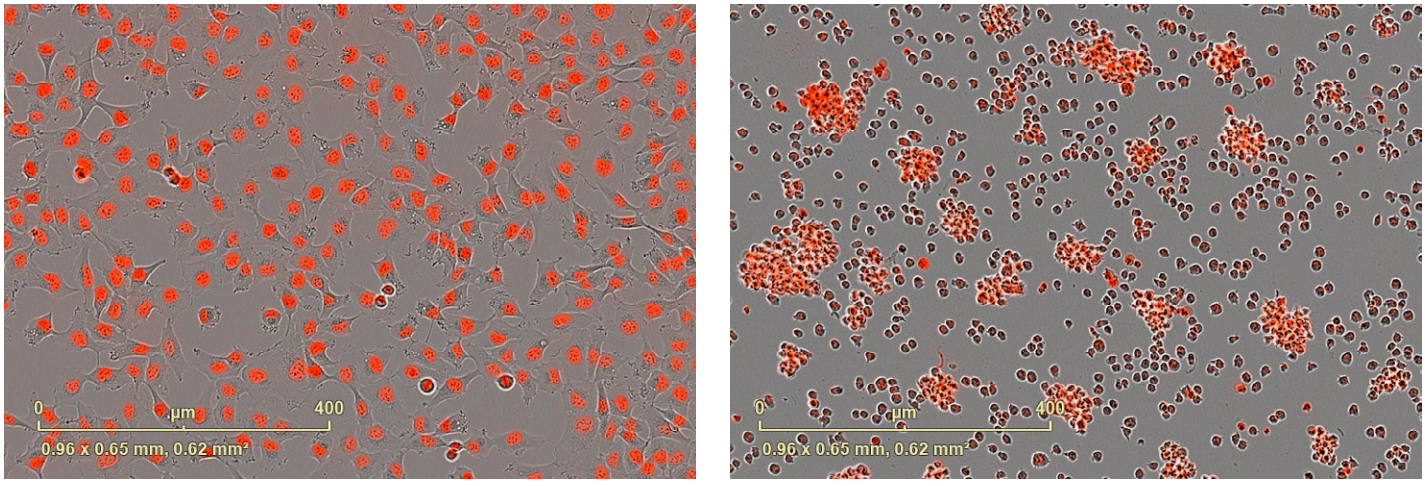
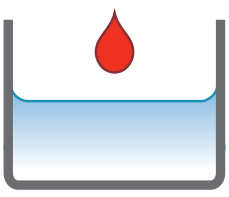


Figure 1: Representative images of fibrosarcoma (HT-1080) and T cell leukemia (Jurkat) cells labeled with the Incucyte® Nuclight Rapid Red Dye. Note the specific DNA labeling of cell nuclei and healthy cell morphology (72 h post cell labeling).

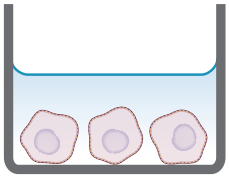
Quick Guide

1. Add reagent



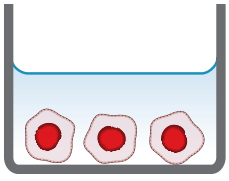
Add Incucyte® Nuclight Rapid Dye

2. Seed cells



Seed cells (50µL/well, 1,000–5000) into a 96-well plate.

3. Live-cell fluorescent imaging



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

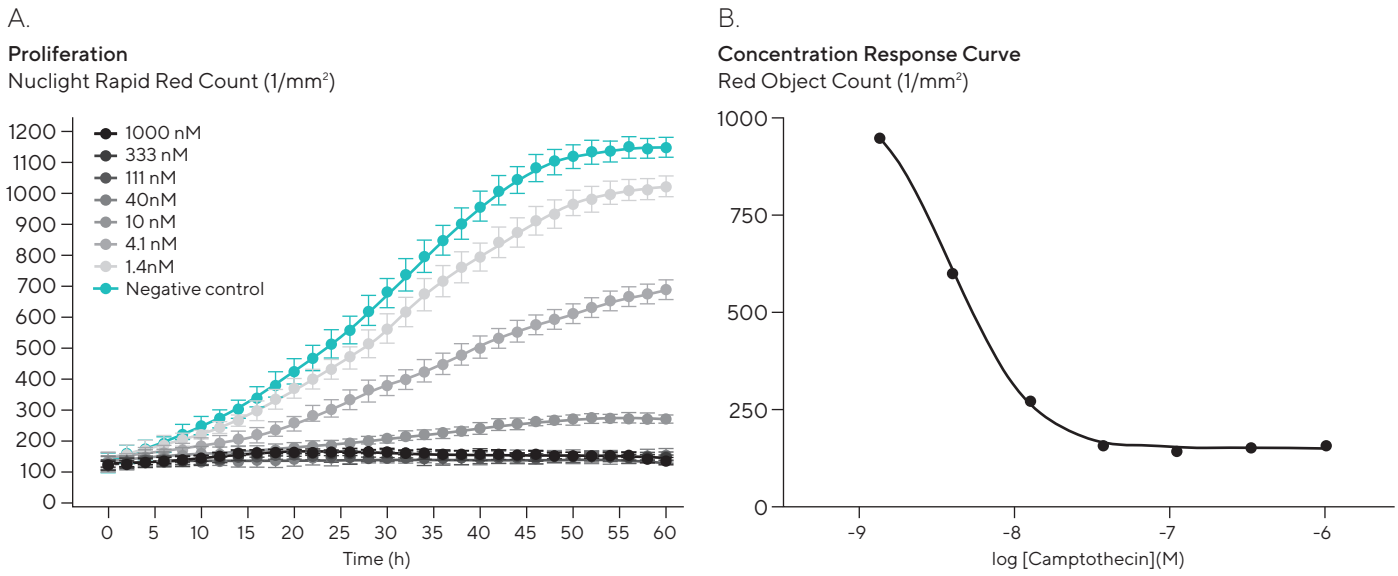


Figure 2: HT-1080 cells labeled with Nuclight Rapid Red were treated with increasing concentrations of Camptothecin and analyzed for Red fluorescent objects over time (A). At 48 hours, Red fluorescent object values were used to calculate the concentration response curve (B).

Protocols and Procedures

Required Materials

- Incucyte® Nuclight Rapid Red Dye
- 0.01% Poly-L-ornithine solution (Sigma Cat. No. P4957)—optional, for non-adherent cells
- Flat bottom tissue culture plate (e.g., Corning Cat. No. 3595)

General Guidelines

- Protect Incucyte® Nuclight Rapid Red Dye 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® 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-) 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 Dye 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.

Preparation and Optimization Assay of Nuclight Rapid Red Dye

1. Prior to harvesting cells, bring one or more vials of Incucyte® Nuclight Rapid Red Dye to room temperature and briefly centrifuge to ensure the reagent is located in the bottom of the vial.
2. For initial cell labeling optimization, dilute the stock to yield a 2X. Working concentration in complete media. To yield a working dilution of 1:125, add 3 µL of Nuclight Rapid Red Dye to 372 µL of complete media.
3. Perform a 2-fold serial dilution of the Nuclight Rapid Red Dye working stock (180 µL of the 1:125 dilution to 180 µL complete media) to create 5 test concentrations in triplicate.

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 types tested.

Note: 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.

To monitor labeling efficiency of individual cells, use the Incucyte® Cell-by-Cell Analysis Software Module (Cat. No. 9600-0031).

For further details of this analysis module and its application see: www.essenbioscience.com/cell-by-cell

Nuclight Rapid Red Cell Labeling Protocol

1. Dilute Nuclight Rapid Red Dye in complete media at the dilution previously determined in the optimization assay, and protect from light (refer to Preparation and Optimization Assay above).
2. Harvest cells using a suitable dissociation solution, then neutralize with complete media.
3. Count and prepare cells at desired density (20,000 cells/mL–100,000 cells/mL, depending on cell type) for assay.
4. Add 50 µL of diluted Nuclight Rapid Red Dye to the desired wells.
5. Mix cell suspension to ensure homogenous mixture, then add 50 µL (final cell density of 1,000–5,000 cells/well) to the wells containing Nuclight Rapid Red Dye.
6. Allow cells to settle at room temperature for 30 minutes before placing the plate in Incucyte® Live-Cell Analysis System.

   Note: A 50 µL/well seeding volume is recommended in order to accommodate the volume of Nuclight Rapid Red Dye.

   Note: Adding the labeling reagent before adding cells will maintain homogenous cell seeding in the wells.

   Note: 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.

   When performing multiplexed functional assays, dilute the Nuclight Rapid Red Dye and Incucyte® Cell Health Reagent (e.g., Caspase-3/7, Annexin V or Cytotox Dyes) ± treatments in complete media to ensure a final assay concentration of 1X. Aspirate media from plate containing cells, and immediately add diluted reagents ± treatments.

7. Place the plate into the Incucyte® Live-Cell Analysis System to monitor nuclear counts using the Red channel. If adding a cell health reagent, onset of cell death via the reagent should also be monitored using all three channels (Phase + Red channel + Cell health fluorescent channel).
   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

Depending on cell types, Incucyte® Nuclight Rapid Red Dye 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

The optimal concentration of Incucyte® Nuclight Rapid Red Dye is the highest concentration that does not cause significant changes to growth rate or morphology while providing efficient cell labeling compared to the unlabeled control. This can be monitored using HD phase images and confluence metrics. Alternatively, Cell-by-Cell Analysis can be used to assess labeling efficiency of your cells. If multiplexing, it is important that spectral un-mixing is used in order to reduce spectral bleed-through from the red to the green channel. This provides for successful masking with our cell toxicity reagents when combined with Nuclight Rapid Red Dye.

Find more information at www.sartorius.com/incucyte

For Research Use Only. Not For Therapeutic or Diagnostic Use.

Sales and Service Contacts

For further contacts, visit www.sartorius.com

Essen BioScience, A Sartorius Company
www.sartorius.com/incucyte
E-Mail: AskAScientist@sartorius.com

Specifications subject to change without notice.
© 2020. All rights reserved. Incucyte®, Essen Bioscience® and all names of Essen Bioscience products are registered trademarks and the property of Essen Bioscience unless otherwise specified. Essen Bioscience is a Sartorius Company. Publication No.: 8000-0724-B00
Status: 08 | 2020

North America
Essen BioScience Inc.
300 West Morgan Road
Ann Arbor, Michigan, 48108
USA
Telephone +1 734 769 1600
E-Mail: orders.US07@sartorius.com

Europe
Essen BioScience Ltd.
Units 2 & 3 The Quadrant
Newark Close
Royston Hertfordshire
SG8 5HL
United Kingdom
Telephone +44 1763 227400
E-Mail:
euorders.UK03@sartorius.com

APAC
Essen BioScience K.K.
4th Floor Daiwa Shinagawa North Bldg.
1-8-11 Kita-Shinagawa
Shinagawa-ku, Tokyo
140-0001
Japan
Telephone: +81 3 6478 5202
E-Mail: orders.US07@sartorius.com