SARTORIUS

Product Guide

Incucyte® Cytolight Rapid Dyes

For Live-Cell Cytoplasmic Labeling

Product Information

Presentation, Storage and Stability

Incucyte® Cytolight Rapid Dyes are supplied as dry powders in sufficient quantity capable of labeling between 10 and 90 million cells depending on the optimized concentration. The dry powder should be stored desiccated at -20° C. Once solubilized with DMSO, the solution should be stored at -20° C and protected from light. When stored as described, the dry powder will be stable for 1 year from date of receipt and the reconstituted solution for at least 1 month.

Product Name	Cat. No.	Ex. Max	Em. Max	Amount	Storage	Stability
Compatible with Incucyte® Live-Cell Analysis Systems configured with a Green | Orange | NIR or Green | Red Optical Module						
Incucyte® Cytolight Rapid Green Dye	4705	492 nm	517 nm	15 µg	-20° C	Powder—1 year from date of receipt Reconstituted—1 month
Compatible with Incucyte® Live-Cell Analysis Systems configured with a Green | Red Optical Module						
Incucyte® Cytolight Rapid Red Dye	4706	630 nm	660 nm	5 x 50 µg	-20° C	Powder—1 year from date of receipt Reconstituted—1 month
Compatible with Incucyte® Live-Cell Analysis Systems configured with a Green | Orange | NIR or Orange | NIR Optical Module						
Incucyte® Cytolight Rapid Orange Dye	4839	546 nm	580 nm	1 mg	-20° C	Powder—1 year from date of receipt Reconstituted—1 month

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

Background

Incucyte® Cytolight Rapid Dyes are stable fluorescent reagents that freely pass through cell membranes; however, once inside, these dyes are transformed into cell-impermeant fluorescent probes that are transferred by dilution to daughter cells but not to adjacent cells within a population. Addition of the Incucyte® Cytolight Rapid Dyes at optimal concentrations to healthy cells is non-perturbing to cell growth or morphology and remains well-retained in cells.

Incucyte® Cytolight Rapid Dyes are ideal for identifying and monitoring cells in mixed cultures up to 48 hours post-labeling. These reagents can be multiplexed with Incucyte® Annexin V, Caspase-3/7 or Cytotox Dyes for simultaneous readouts of apoptosis or cytotoxicity using the Incucyte® Live-Cell Analysis System.

Please note that the intensity of the Cytolight Rapid Dye signal will decrease over the course of the assay as it is diluted between daughter cells. For this reason, measurements beyond 48 hours post-labeling are generally not recommended. These reagents are also not recommended for measuring proliferation in fast growing cells over a long period of time as the rapid decline in fluorescence, due to reagent dilution between daughter cells, can impair fluorescence area and count metrics.

Example Data

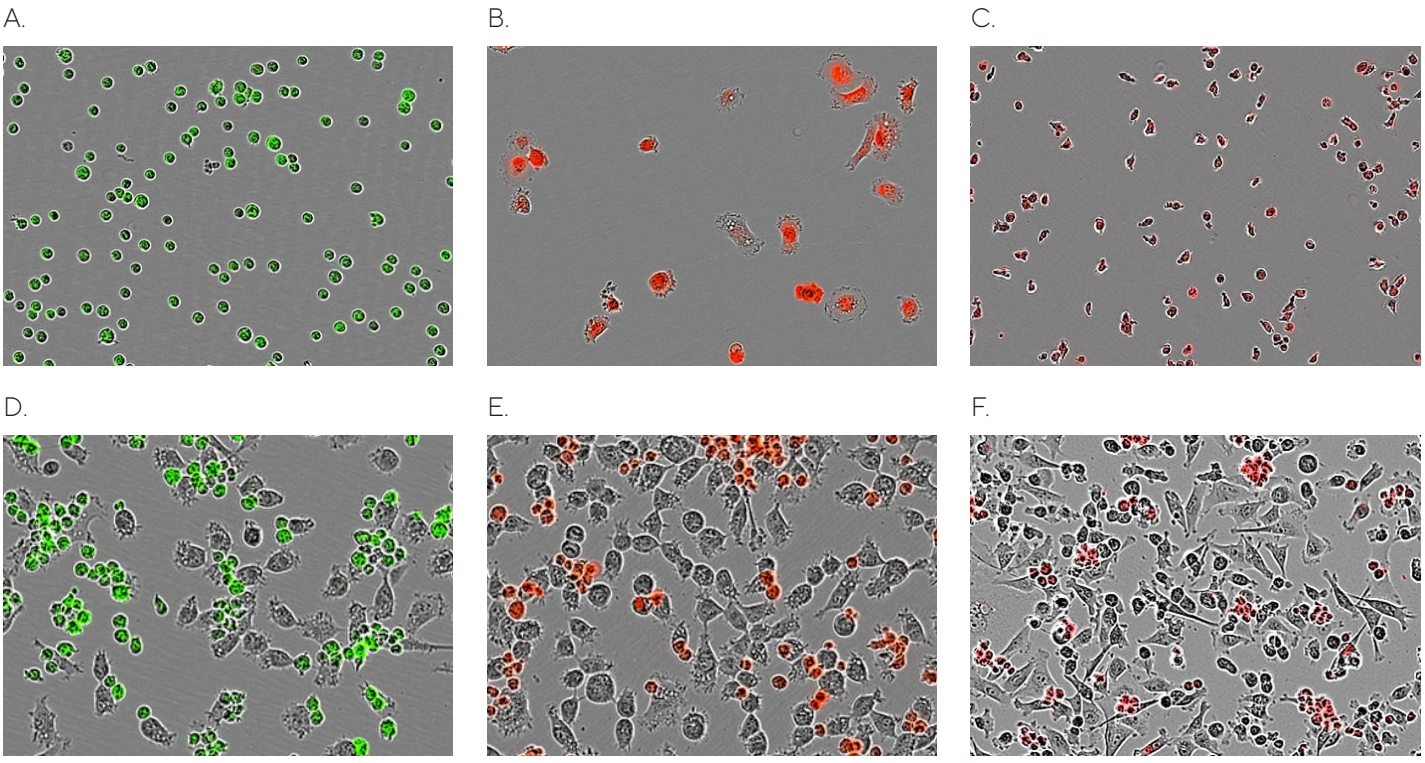


Figure 1: Incucyte® Cytolight Rapid Dyes enable rapid, high efficiency labeling of cells, and are ideal for use in co-culture models. (A) Jurkat cells labeled with Incucyte® Cytolight Rapid Green. (B) HT-1080 cells labeled with Incucyte® Cytolight Rapid Red. (C) Human peripheral blood mononuclear cells (PBMCs) labeled with Incucyte® Cytolight Rapid Orange. (D-F) Incucyte® Cytolight Rapid Green (D), Red (E), or Orange (F) labeled Jurkat cells co-cultured with HT-1080 cells. Strikingly different morphologies between the labeled, non-adherent Jurkat cells and non-labeled, adherent HT-1080 cells enabled visual inspection of the images indicating little or no cell-to-cell transfer.

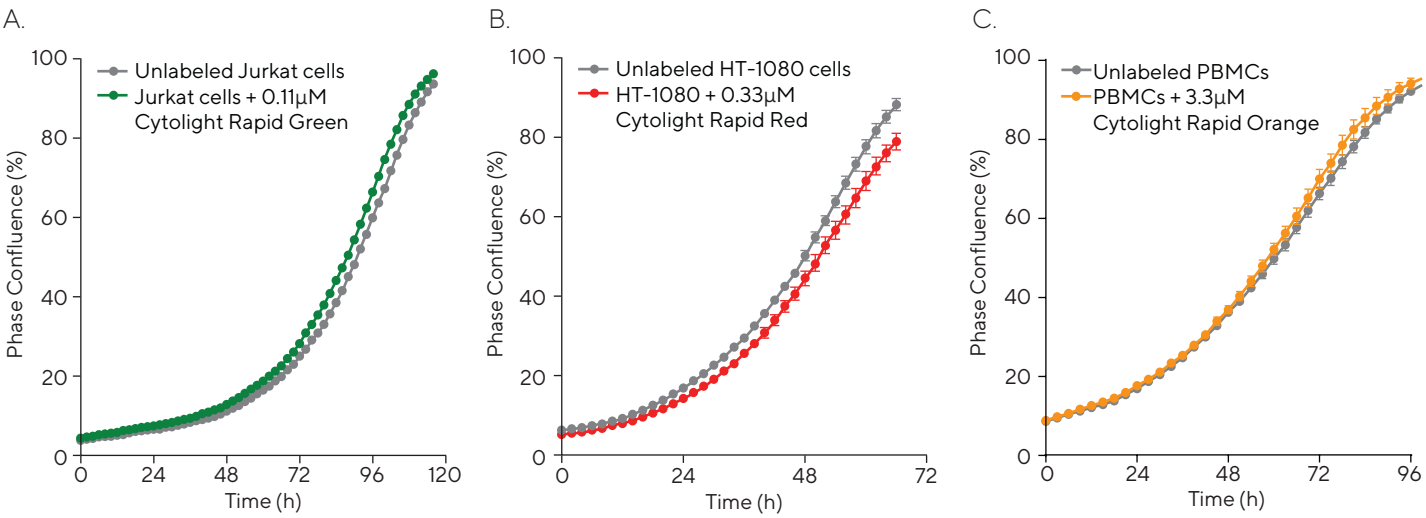


Figure 2: Incucyte® Cytolight Rapid Dyes are non-perturbing to non-adherent and adherent cell lines and primary cells. Phase contrast image analysis was used to determine proliferation time-courses for (A) Jurkat cells (non-adherent), (B) HT-1080 cells (adherent), or (C) PBMC (primary cells) labeled with optimized concentrations of Incucyte® Cytolight Rapid Green (0.11 µM), Red (0.33 µM) or Orange (3.3 µM) Dye, respectively. Data is presented as the mean ± SEM from 4 replicate wells.

Recommended Use

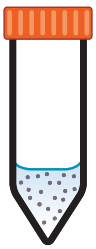
Solubilize Incucyte® Cytolight Rapid Dyes with high-quality DMSO to prepare stock solutions as listed in Table 1. The reagents may then be diluted in phosphate-buffered saline for labeling cells. Labeling concentrations for the reagents will need to be optimized for each cell type to avoid toxicity and to ensure that the fluorescent signal remains detectable for the duration of your experiment. We recommend making this assessment by testing a range of concentrations of the dye and using Incucyte® integrated analysis software confluence and fluorescence metrics to identify the optimal concentration that yields a sufficient signal with minimal cell growth perturbation within your experiment. Table 1 lists the working concentration range for each dye. When used with an Incucyte® Live-Cell Analysis System, we recommend data collection every 1–3 hours.

Table 1: Solubilization of Incucyte® Cytolight Rapid Dyes

Product Name	Cat. No.	Vials Supplied	DMSO per Vial	Stock Concentration	Working Concentration
Incucyte® Cytolight Rapid Green Dye	4705	1	21.5 µL	5 mM	0.11–0.33 µM
Incucyte® Cytolight Rapid Red Dye	4706	5	20 µL	1 mM	0.33–1 µM
Incucyte® Cytolight Rapid Orange Dye	4839	1	380 µL	5 mM	2.5–10 µM

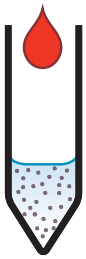
Quick Guide

1. Harvest cells



Harvest cells. Wash with PBS. Count and resuspend in PBS (1×10^5 cells/mL)

2. Label

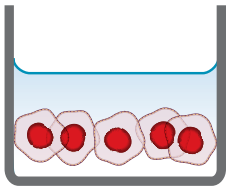


Add Incucyte® Cytolight Rapid Dye. Incubate for 20 minutes at 37° C.

3. Bind excess reagent

Bind excess dye by adding complete medium. Centrifuge and aspirate supernatant.

4. Live-cell imaging



Resuspend cells in complete medium and seed at desired density. Acquire images every 1–3 hours with Incucyte® Live-Cell Analysis System.

Protocols and Procedures

Required Materials

- Incucyte® Cytolight Rapid Green Dye (Sartorius Cat. No. 4705) or
- Incucyte® Cytolight Rapid Red Dye (Sartorius Cat. No. 4706) or
- Incucyte® Cytolight Rapid Orange Dye (Sartorius Cat. No. 4839)
- 96-well flat bottom microplate (e.g., Corning Cat. No. 3595)
- 0.01% Poly-L-ornithine solution (Sigma Cat. No. P4957)
  - Optional, for non-adherent cells

General Guidelines

- Protect Incucyte® Cytolight Rapid Dye from light at all times.
- We recommend medium with low levels of riboflavin to reduce the green fluorescence background. EBM, F12-K, and Eagles MEM have low riboflavin (< 0.2 mg/L). DMEM and RPMI have high riboflavin (> 0.2 mg/L).
- 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 (not supplied) to prevent cell aggregation at well edges. For a 96-well plate, add 50 µL of poly-L-ornithine solution to each well, incubate for 1 hour at ambient temperature, remove solution from wells, and 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® Cytolight Rapid Dye when labeling cells. To determine this concentration, perform an initial optimization experiment as described below each time when a new cell type is used.
- For best labeling efficiency, prepare 100X working solution freshly with ambient temperature PBS. Keep the solution at room temperature and use it within 1 hour post-preparation. Pre-warming the 100X working solution to 37°C before addition to cells is not recommended.
- If fluorescence debris is observed in Cytolight Orange labeled cells, add Tris-HCl (pH 7.4) to a final concentration of 50 mM and incubate for 5 minutes at 37° C before adding culture medium in Step 3.1.

Preparation of Incucyte® Cytolight Rapid Dye

1. Prepare Stock Concentration

1.1 Prior to harvesting cells, bring vials of Incucyte® Cytolight Rapid Dye to room temperature. Briefly centrifuge to ensure the dye is collected at the bottom of the vial.

1.2 Solubilize Cytolight Rapid Dye with high-quality DMSO (Table 1).

2. Prepare Working Concentration

2.1a General Use

Dilute the stock solution in PBS (Ca^{2+}/Mg^{2+}-free) to yield a 100X working concentration (e.g., prepare a 100 µM solution for final assay concentration of 1 µM). Use within 1 hour.

Note: Use PBS stored at room temperature.

2.1a Initial Optimization

When performing initial cell labeling optimization, dilute the stock solution in PBS (Ca^{2+}/Mg^{2+}-free) to yield a 100X working concentration.

Note: Use PBS stored at room temperature. Use Table 1 as reference to choose the starting 100X working concentration.

2.2 Initial Optimization

Perform a 2- or 3-fold serial dilution of the Cytolight Rapid Dye 100X working stock. Be sure to prepare enough volume for 10 µL per 1 mL labeling reaction. Use within 1 hour.

Cell Labeling Protocol

1. Harvest Cells

1.1 Harvest cells using a suitable dissociation solution, then neutralize with full growth media and centrifuge (500 x g, 5 minutes) to create a pellet.

Note: Grow enough cells in advance to accommodate the different conditions required to set up the experiment (refer to step 4.1 for recommended seeding densities). It is recommended to prepare 50% more cells than needed in order to accommodate for cell loss during the wash step with PBS.

1.2 Aspirate the supernatant and wash cells with ambient temperature PBS (Ca^{2+}/Mg^{2+}-free).

1.3 Centrifuge cells (500 x g, 5 minutes) and aspirate the supernatant.

1.4 Resuspend the cell pellet in PBS (Ca^{2+}/Mg^{2+}-free) and determine the cell concentration. Adjust to yield a final cell density of 1×10^5 cells/mL.

2. Label Cells

2.1 Add 10 µL 100X working solution of Cytolight Rapid Dye per mL of cell suspension, mix thoroughly.

2.2 Incubate for 20 minutes at 37° C, mixing gently every 10 minutes.

3. Bind Excess Dye

3.1 Bind excess dye by adding a 6-fold volume of serum-containing cell culture medium to the labeling reaction (e.g., add 6 mL to 1 mL cell/dye suspension).

3.2 Centrifuge (500 x g, 5 minutes) and aspirate the supernatant completely to remove residual fluorescence debris from the labeled cells.

Note: Complete removal of culture media from the pellet is essential for the success of this assay when using Cytolight Rapid Orange Dye.

4. Seed Cells

4.1 Resuspend cells to the desired density in complete cell culture medium and seed the labeled cells at an appropriate density into a 96-well plate. The seeding density will need to be optimized for the cell line used; however, we have found that 2,000–5,000 cells per well for adherent cell types or 20,000–30,000 cells per well for non-adherent cell types are reasonable starting points.

4.2 Allow cells to settle at ambient temperature for 30 minutes.

5. Live-Cell Imaging

Place the plate into the Incucyte® Live-Cell Analysis System to monitor both cell proliferation using the phase contrast and labeling efficiency using the appropriate fluorescence channel.

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

Evaluating Results to Determine Optimal Dye Concentration

Use the Incucyte® HD phase contrast images and confluence metrics to compare the cell morphology and growth rates for each concentration of dye and compare to the non-labeled control cells. The optimal concentration of Incucyte® Cytolight Rapid Dye is the concentration that does not cause significant changes to growth rate or morphology while providing efficient cell labeling.

Please note that you may find that there is some variability in the initial confluence values for cells at different dye concentrations. This is due to the independent preparation and seeding of each assay condition. We recommend normalizing the confluence values for each test condition to the first scan. Comparison between the fold-increase in confluence over time can then be used to identify optimal dye concentration.

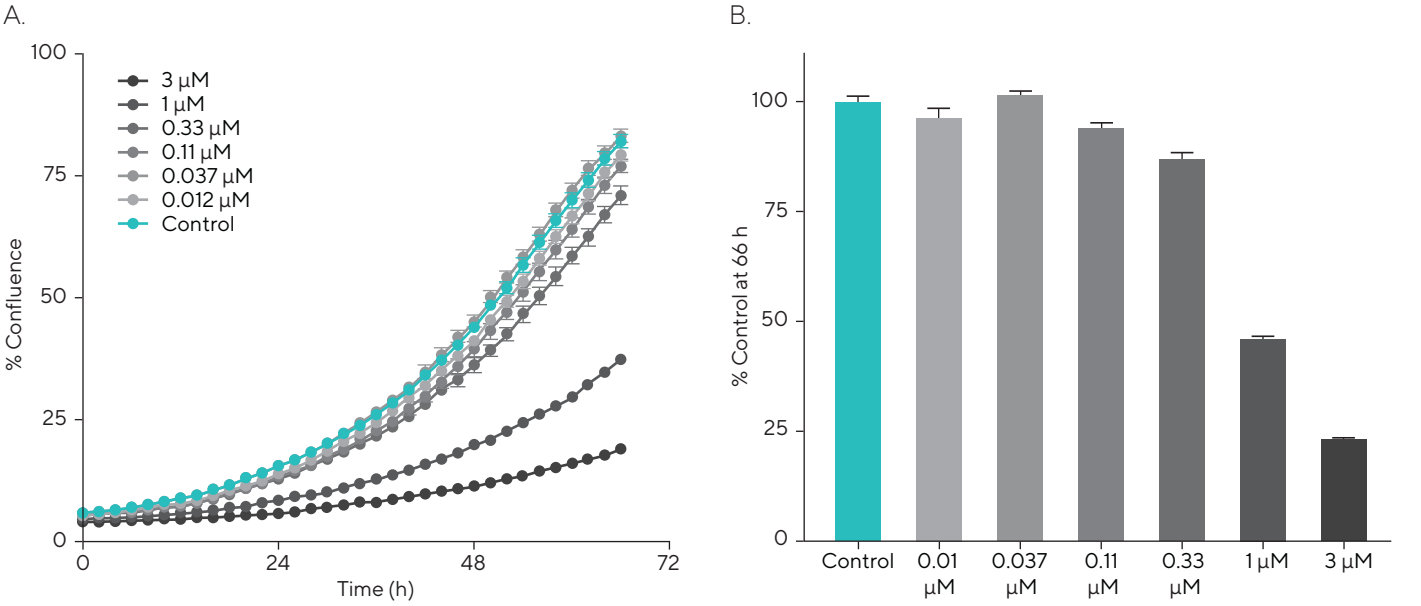


Figure 3: Selecting the optimal dye concentration. Incucyte® HD phase contrast image analysis was used to determine (A) cell growth time-courses and (B) % of control values for HT-1080 (adherent) cells labeled with the Incucyte® Cytolight Rapid Green Dye. Data is presented as the mean ± SEM from 6 replicate wells. In this experiment, the optimal dye concentration was 0.11 µM.

Optional: Labeling Efficiency

A labeling efficiency can be calculated on the Incucyte® Live-Cell Analysis System using the Incucyte® Cell-by-Cell Analysis Software Module (Cat. No. 9600-0031).
This enables individual cell identification and subsequent classification into subpopulations based on properties including fluorescence intensity. These subpopulations can then be expressed as a percentage of the total population being labeled. To use this module the following settings should be used:

a. Scan type: Standard | Adherent or Non-Adherent Cell-by-Cell
b. Objective: 10X (for adherent cells) or 20X (for non-adherent cells)

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

Multiplexing Optimization

When multiplexing with multiple fluorescent reagents, spectral unmixing may be required to account for signal that has been contributed from one of the given channels. Spectral unmixing values must be applied prior to running an analysis job.

- **Cytolight Rapid Green**: No spectral unmixing required
- **Cytolight Rapid Red**: 0.5% recommended to remove Red contributing to Green
- **Cytolight Rapid Orange**: 5-6% recommended to remove Orange contributing to Green

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

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

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