IncuCyte® CytoLight Rapid Reagents for Live-Cell Labeling

Catalog numbers: 4705 and 4706

Presentation, storage and stability

IncuCyte® CytoLight Rapid Reagents are supplied as dry powder in sufficient quantity capable of labeling between 10 and 100 million cells depending on the optimized concentration. The dry powder should be stored desiccated at -20°C and once solubilized, the solution should be stored at -20°C and protected from light. When stored as described, the dry powder will be stable until indicated expiry date and the reconstituted solution for at least 1 month.

Background and intended use

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

The IncuCyte CytoLight Rapid Reagents 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 reagents for simultaneous readouts of apoptosis or cytotoxicity using the IncuCyte® live-cell analysis system.

Please note that the intensity of the CytoLight Rapid Reagent 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 rapidly growing cells over long periods of time as the steady decline in fluorescence, due to reagent dilution between daughter cells, can impair fluorescence area and count metrics.

Reconstitution and recommended use

Solubilize the IncuCyte CytoLight Rapid Reagents by adding high-quality DMSO to prepare final concentrations 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 reagent-related 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 (0.03 – 3 µM) and using the IncuCyte system confluence and fluorescence metrics to identify the lowest concentration of CytoLight Rapid Reagent that yields a sufficient signal within your experiment. When used with an IncuCyte® live-cell analysis system we recommend data collection every 1-3 hours.

For further information of labeling, please see the relevant protocol published on our website: **essenbioscience.com/CytoLightRapid**

Safety data sheet (SDS) information

Download the SDS from our website: **essenbioscience.com/CytoLightRapid**

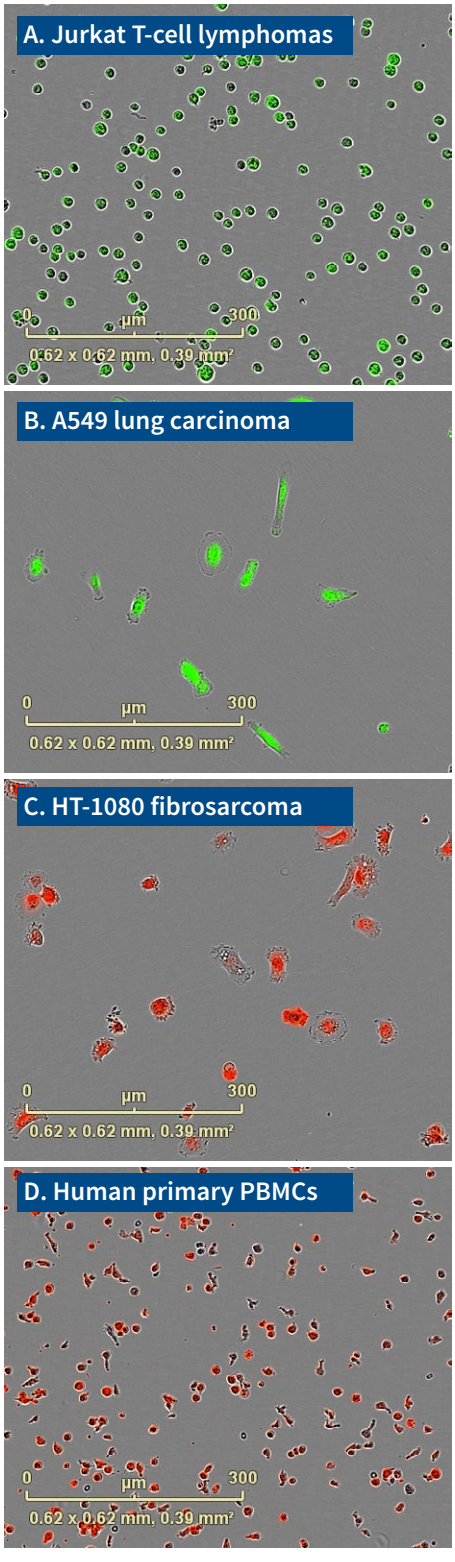


Figure 1. IncuCyte CytoLight Rapid Reagents enable rapid, high efficiency labeling of adherent and non-adherent primary cells and cell lines.
Images captured using an IncuCyte® live-cell analysis system within 30 minutes of the labeling procedure show high efficiency cell labeling (>95%). **(A)** Jurkat T-cell lymphoma, **(B)** A549 lung carcinoma, **(C)** HT-1080 fibrosarcoma and **(D)** human primary peripheral blood mononuclear cells (PBMCs). Note the homogeneous cytoplasmic labeling and the healthy cell morphology.

Table 1. Solubilization of IncuCyte CytoLight Rapid Reagents

Product	Cat No.	Vials Supplied	Volume of DMSO per Vial	Stock Concentration
IncuCyte® CytoLight Rapid Green Reagent	4705	1	21.5 µL	5 mM
IncuCyte® CytoLight Rapid Red Reagent	4706	5	20.0 µL	1 mM

Quick guide

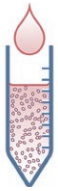
1 HARVEST CELLS

Harvest cells. Wash with dPBS. Count and resuspend in dPBS (1 x10^5 cells/ml).

2 LABEL

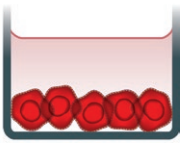
Add IncuCyte® CytoLight Rapid live-cell labeling reagent. Incubate for 20 minutes at 37 °C.

3 BIND EXCESS REAGENT



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

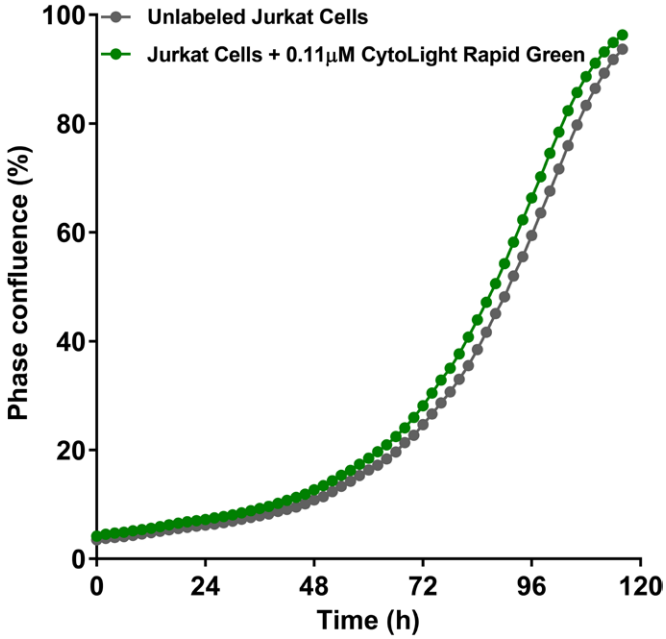
4 LIVE-CELL FLUORESCENT ANALYSIS



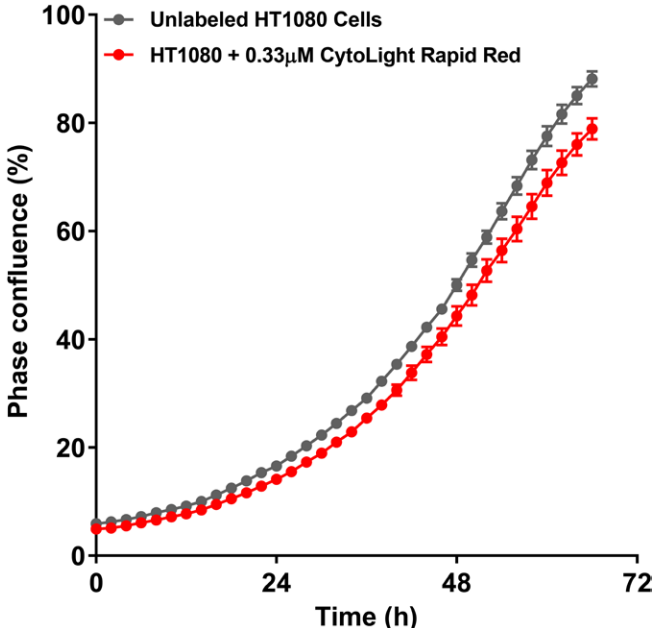
Resuspend cells in complete medium and seed at desired density. Acquire images every hour (10x or 20x) in the IncuCyte® System.

Figure 2. IncuCyte CytoLight Rapid Reagents are non-perturbing to non-adherent and adherent cell types.

A IncuCyte CytoLight Rapid Green Reagent



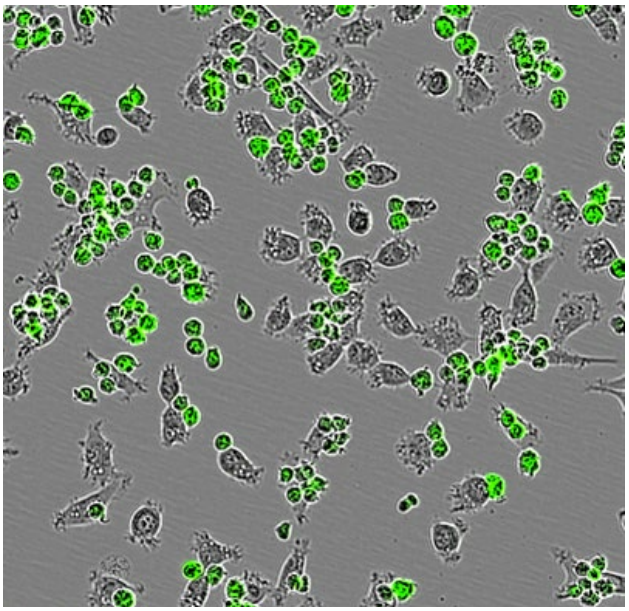
B IncuCyte CytoLight Rapid Red Reagent



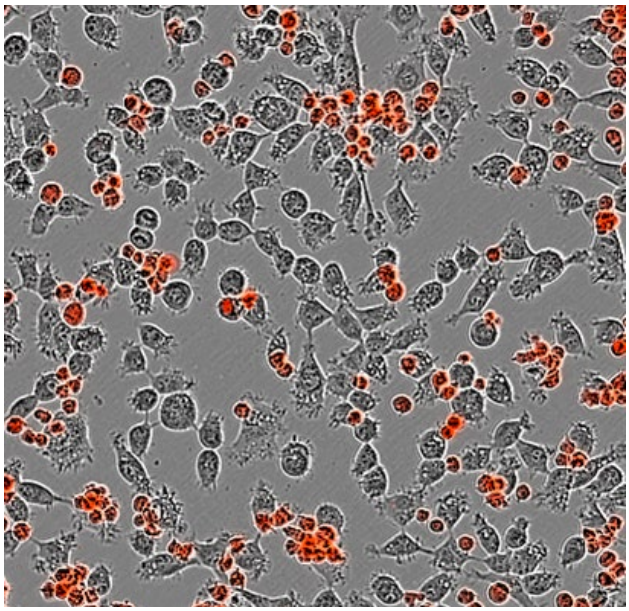
Phase contrast image analysis was used to determine proliferation time-courses for **(A)** Jurkat cells (non-adherent) or **(B)** HT-1080 (adherent) cells labeled with optimized concentrations of CytoLight Rapid Green or Red Reagents (0.11µM and 0.33µM) respectively. Data is presented as the mean ± SEM from 4 replicate wells.

Figure 3. IncuCyte CytoLight Rapid Reagents do not transfer to neighboring cells making them ideal for use in co-culture models.

A IncuCyte CytoLight Rapid Green Reagent



B IncuCyte CytoLight Rapid Red Reagent



(A) IncuCyte CytoLight Rapid Green and **(B)** IncuCyte CytoLight Rapid Red Reagent 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.

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE.

Product	Cat No.	Amount	Ex. maxima	Em. maxima
IncuCyte CytoLight Rapid Green Reagent	4705	100-1000 tests	492 nm	517 nm
IncuCyte CytoLight Rapid Red Reagent	4706	100-1000 tests	630 nm	660 nm