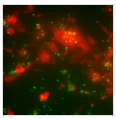

Oct 14, 2019

Staining cells with IncuCyte Cytolight Rapid Dyes for flow cytometry or fluorescent microscopy

Elinor Gottschalk[1], Bulent Arman Aksoy[1], Pinar Aksoy[1], Eric Czech[1], Jeff Hammerbacher[1]

[1]Medical University of South Carolina

1 Works for me dx.doi.org/10.17504/protocols.io.73nhqme

Hammer Lab
Tech. support phone: **+18437924527** email: **arman@hammerlab.org**

Elinor Gottschalk

ABSTRACT

This protocol is for labeling cells with IncuCyte Cytolight Rapid dyes from Essen Bioscience. We optimized the dye concentration for Jurkat cells, pmel-1 T cells, and B16F10 melanoma cells.

GUIDELINES

We optimized IncuCyte® CytoLight Rapid Green Reagent for live cell cytoplasmic labeling with Jurkat cells, pmel-1 T cells, and IncuCyte® CytoLight Rapid Red Reagent with B16F10 melanoma cells. The optimal concentrations (based on minimal decrease in cell viability and highest percentage of stained cells) were:

- Jurkat: 1 µM (green)
- pmel-1 T cells: 0.11 µM (green)
- B16F10 cells: 0.11 µM (red)

We visualized the stained cells by flow cytometry or on the Keyence BZ-X710 fluorescence microscope equipped with GFP and Cy5 filters.

MATERIALS

NAME	CATALOG #	VENDOR
IncuCyte® CytoLight Rapid Green Reagent for live cell cytoplasmic labeling	4705	Essen Biosciences
1X PBS	75800-986	VWR Scientific
IncuCyte® CytoLight Rapid Red Reagent for live cell cytoplasmic labeling	4706	Essen Biosciences

1 Harvest cells and wash with 1X PBS

2 Centrifuge **350 x g 5 minutes**

2.1 Resuspend cells in PBS at 1 million cells per ml

3 Add IncuCyte Dye
- For pmel-1 T cells: we determined that 0.11 µM of green dye was optimal
- We made our working stock 11 µM (so for every 1 ml of cell suspension, we add 10 ul of working stock of dye)
- For Jurkat cells, 1 µM of green dye was optimal
- For B16F10 cells, 0.11 µM of red dye was optimal

3.1 Incubate the cells at **37 °C** for **00:20:00** (we wrapped the tube in foil and placed it in a water bath).
- invert the tube twice during incubation to mix the cells

3.2 Add a 6-fold volume of complete media (containing serum) to bind the excess dye.

3.3 Centrifuge **350 x g** **5 minutes**

4 Resuspend cell in complete media at the desired concentration.
- Two different cell types can be labeled with different dyes and then co-cultured and visualized
- Stained T cells can be visualized migrating in collagen gel

5 Assay the cells via flowcytometry or fluorescence microscopy.
Flow cytometry: FITC for green dye; APC for red dye
Fluorescence microscopy: FITC filter for green dye; Cy5 filter for red dye