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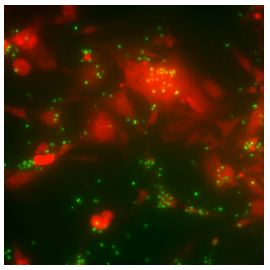
Staining cells with IncuCyte Cytolight Rapid Dyes for flow cytometry or fluorescent microscopy



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We use this protocol and it's working

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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

MATERIALS

IncuCyte® CytoLight Rapid Green Reagent for live cell cytoplasmic labeling **Essen Biosciences Catalog #**4705

1X PBS **VWR International (Avantor) Catalog #**75800-986

IncuCyte® CytoLight Rapid Red Reagent for live cell cytoplasmic labeling **Essen Biosciences Catalog #**4706

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