Flow Post-its
Barbara Oliveira
Kathy Daniels

Cell Proliferation by Flow Cytometry

March 2020

In Cancer Research, assessing **Cell Proliferation** can be useful for a variety of applications:

Cytotoxicity assays

CAR-T cell expansion

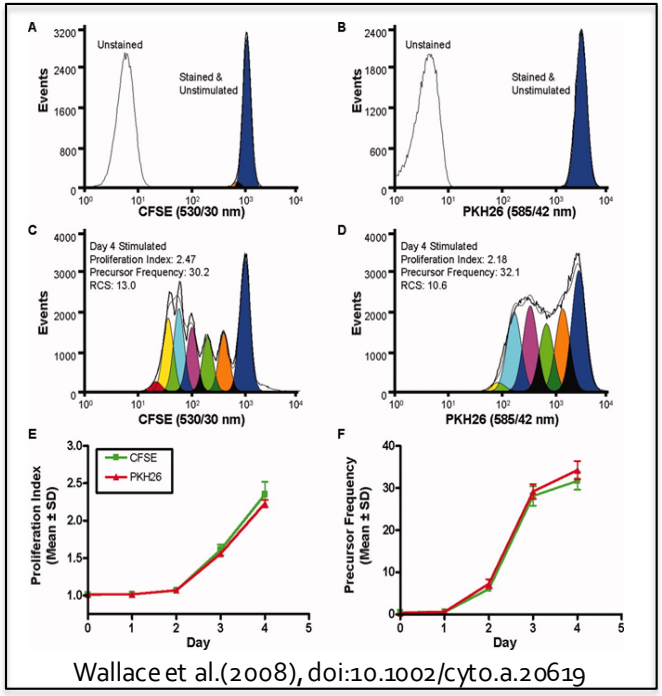
Drug Development

Determining inhibition of tumor growth

Tips for Optimizing Your Experiment

- Determine best time points for acquisition.
- Optimize reagent concentration. Excess dye can result in cell death.
- Use division index or proliferation index to analyze data. This can be calculated across various analysis platforms via modeling.
  Roederer (2011), doi:10.1002/cyto.a.21010
- Include absolute cell count.

How? Cell proliferation can be assessed using multiple approaches.



Wallace et al.(2008), doi:10.1002/cyto.a.20619

1) Nucleoside-analog incorporation during DNA synthesis (e.g. BrdU or EdU)

As DNA is synthesized and cells divide, a thymidine nucleoside analogue (BrdU/EdU) is incorporated and subsequent staining with a fluorescent labeled anti-BrdU/EdU allows for detection of proliferating cells.

2) Dye dilution proliferation assays (e.g. CFSE, CellTrace or PKH67)

CFSE & CellTrace covalently bind to intracellular molecules, such as amines while PKH67 is a lipophilic membrane intercalating dye. Dyes of this group remain in cells, and as they divide, each daughter cell ends up with half the amount of dye.

3) Labeling cell cycle-associated proteins (e.g. Ki-67, MCM2, PCNA)

Specific markers are expressed by cells as they are undergoing cell division. Fluorescently tagged antibodies specific to these proteins can be used in flow cytometry experiments to detect actively dividing cells.

EdU: 5-ethylnyl-2'-deoxyuridine; **BrdU**: bromodeoxyuridine; **CFSE**: Carboxyfluorescein succinimidyl ester
MCM2:minichromosome maintenance **PCNA**: Proliferating cell nuclear antigen