

Sep 29, 2025

Cell Cycle Analysis by Flow Cytometry

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DOI

<https://dx.doi.org/10.17504/protocols.io.kqdg3176el25/v1>

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Protocol Citation: Maria Jose Perez J. 2025. Cell Cycle Analysis by Flow Cytometry. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.kqdg3176el25/v1>

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Protocol status: Working

We use this protocol and it's working

Created: September 25, 2025

Last Modified: September 29, 2025

Protocol Integer ID: 228168

Keywords: flow cytometry cell cycle analysis

Funders Acknowledgements:

ASAP

Abstract

Cell Cycle Analysis by Flow Cytometry



- 1 Dissociate iPSC-derived cells using Accutase and resuspend cells in PBS.
- 2 Stain cells with Hoechst 33342 at 2.5 µg/mL for 30 minutes at 37°C.
- 3 Wash cells with PBS
- 4 Incubate cells with Zombie NIR viability dye at 1:1000 dilution in PBS to exclude dead cells
- 5 Wash cells with PBS.
- 6 Resuspend cells in PBS containing 2% fetal bovine serum (FBS).
- 7 Transfer cells into 5 mL round-bottom tubes for acquisition
- 8 Perform flow cytometry on a Sony ID7000TM spectral cell analyzer (Sony Biotechnology, USA) at a maximum event rate of 200 events per second.
- 9 Analyze acquired data using the cell cycle analysis module in FlowJoTM software (BD Biosciences).
- 10 Apply the Watson (Pragmatic) model for cell cycle quantification.
- 11 Distinguish G1, S, and G2/M phases based on Hoechst signal intensity (G1 in red, S in light gray, G2/M in blue).
- 12 Visualize model fitting by overlaying the model sum (dark gray) and actual signal distribution (black line).
- 13 Constrain parameters by setting Peak 1 to "n" and constraining Peak 2 to match the coefficient of variation (CV) of the G1 peak.

- 14 Select the final model based on achieving a low root mean square deviation (RMSD) value.