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Cell Cycle Analysis by Flow Cytometry

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We use this protocol and it's working

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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 ID7000™ 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 FlowJo™ 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.