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TH-TdTomato expression measurement by FACS

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We use this protocol and it's working

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Abstract

This protocol outlines the preparation and flow cytometry-based analysis of midbrain neurons differentiated from Td-Tomato reporter hPSCs.

Cell Culture and Preparation

1 Differentiate TH-TdTomato lines into midbrain neurons in **6-well plates** until the desired **differentiation day**.

2 Confirm cell morphology and fluorescence (if applicable) before harvesting.

Cell Detachment

3 Remove media and wash cells once with PBS

4 Gently lift neurons using Accutase supplemented with ROCK inhibitor (Y-27632). 1ml per well of a 6w plate

4.1 Incubate as needed (typically ~5 minutes) at RT until cells detach.

5 Collect cells into tubes with twice the volume of culture medium containing ROCK inhibitor.

6 Centrifuge the cell suspension at 300 × g for 5 minutes at room temperature.

7 Carefully remove the supernatant.

8 Resuspend the pellet in FACS buffer: PBS + 1x B-27 supplement + ROCK inhibitor (Y-27632) + DAPI (final concentration 1µg/ml) for live/dead discrimination

8.1 Keep cells on ice from this step forward

8.2 Make sure to include a sample with no DAPI for proper gating

9 For gating: collect TdTomato-negative line, or undifferentiated iPSC as a negative control to establish the TdTomato gate.

10 Pass the resuspended cells through a 35 µm cell strainer into FACS-compatible tubes

Flow citometry (FACS)

11 Analyze TdTomato fluorescence using a flow cytometer with appropriate excitation/emission settings (e.g., 554/581 nm) and DAPI (Ex/Em ~358/461 nm).

11.1 Record at least 10,000 events from live population

12 Gating Strategy:

12.1 FSC/SSC: Gate to exclude debris and cell clumps.

12.2 DAPI-negative cells: Gate for live cells excluding DAPI+ dead cells

12.3 TdTomato fluorescence:
Set gate using Td-tomato negative line
Identify TdTomato+ neurons and MFI from live population.

13 Analysis with proper software (FlowJo)