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Flow cytometry

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ABSTRACT

This protocol is for immunolabelling fixed midbrain dopaminergic neurons for flow cytometry analysis and downstream flow cytometry acquisition.

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Sample preparation

1 Cells were washed once with PBS

2 The cells were incubated with Accutase (Gibco) to generate a single-cell suspension for 5m

00:05:00 at 37 °C

3 A cell suspension of 500k/ml was prepared in media

4 Cells were then spun down at 200 x g, 00:05:00 , and the supernatant was removed. 5m

5 Cell pellet was resuspended gently in 4ml of 4% paraformaldehyde and briefly vortexed at a low speed before being spun on a rotation spinner for 00:10:00 at room temperature. 10m

6 After fixation, samples were spun down and supernatant removed

7 Cells were resuspended in 2ml of 0.1% BSA (Sigma) in PBS.

8 After resuspension, cells were filtered through a 70µm strainer (Miltenyi Biotec) to filter out any cell clumps

9 Cells were then centrifuged 200 x g, 00:05:00 , and the supernatant was removed. 5m

10 Cell pellets were then resuspended in 1ml of permeabilization/blocking buffer (0.1% Triton X-100, 1% BSA, 10% normal goat serum (Sigma) in PBS), and incubated on a rotation spinner for 00:30:00 at room temperature. 30m

11 After permeabilization/blocking, cells were centrifuged 200 x g, 00:05:00 and the supernatant was removed. 5m

12 Cells were then resuspended in the primary antibodies (1:200) made up in 0.1% BSA in PBS, 1h

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and incubated on the rotation spinner for **01:00:00** at room temperature.

13 After primary antibody incubation, cells were centrifuged **200 x g, 00:05:00** , supernatant removed and washed once in 0.1% BSA in PBS. 5m

14 Cells were then resuspended in the species-specific secondary antibodies (AlexaFluor 488, 647) at a dilution of 1:500 made up in 0.1% BSA in PBS and incubated in the dark on a rotation spinner for **00:30:00** . 30m

15 After incubation, cells were centrifuged **200 x g, 00:05:00** , supernatant removed and washed once in PBS, followed by incubation with DAPI made up in PBS for **00:05:00** . 10m

16 The DAPI + PBS was then removed, followed by one wash in PBS, before being analysed on the flow cytometer.

Cell sorting and analysis

17 The samples were run on the LSRii (BD) cell sorter. Scattering was initially used to discard debris as well as cell doublets and larger clumps.

18 The single-cell population was then gated to include DAPI positive only cells (negative control).

19 The gating threshold for measured channels was determined using the control lacking the antibody of interest (Fluorescence minus one (FMO) control), for both channels being recorded.

20 Once the parameters had been set, 10,000 cell events were recorded, and data were processed and analysed on the FlowJo software.

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