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Fluorescently labeled polyamine uptake (via Flow Cytometry)

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Marine Houdou[1], Peter Vangheluwe[1]

[1]KU Leuven

M Marine Houdou

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Protocol Particulars Video

The video below is a supplement with extra context and tips, as part of the Aligning Science Across Parkinson's (ASAP) Protocol Particulars video interview series, featuring conversations with protocol authors.

https://www.youtube.com/embed/c0GkyegZaG8?si=ZnQ8VCKG_nkCvcng

Abstract

Assess polyamine uptake capacities of a specific cell line after incubation with fluorescently labeled polyamines and mean fluorescence intensitiy acquisition *via* flow cytometry.

Guidelines

Make sure to stay protect from the light when manipulating fluorescent materials.

Materials

0.25% Trypsin-EDTA: Gibco, 25200056

Albumin Fraction V: Carl Roth, 8076.4

Dulbecco's Phosphate Buffered Saline modified without calcium chloride and magnesium chloride (DPBS (-/-)): Gibco, D8537

TrypLE Express Enzyme: Gibco, 12604021

Versene Solution: Gibco, 15040

Before start

- Prepare appropriate dilution of fluorescently labeled polyamine in cell culture medium.
- Prepare Flow Cytometry (FC) buffer made of 1% Albumin Fraction V in DPBS (-/-).
- Prepare Eppendorf tubes and FC tubes by labeling them and keeping them on ice.

1 Seed cells in 12 well-plate that they reach 70%-80% confluency the day of the experiment.

Note

This protocol is made for a 12 well-plate format. Adaptations need to be done to scale up or down.

2 The day of the experiment, remove cell culture medium and add fluorescently labeled polyamines to the cells in a final volume of 500 µL / well .

Note

Concentration and incubation time may vary depending on the cell type. It's best to try different incubation times and doses to better appreciate the kinetics.

Note

Stay protect from the light.

3 Incubate the desired time at 37°C, 5% CO_2, protected from the light.

4 Harvest cells and prepare samples for Flow Cytometry (FC).

4.1 Discard medium.

4.2 Wash with 500 µL /well Versene or DPBS (-/-).

4.3 Discard Versene or DPBS (-/-).

4.4 Add 200 µL /well 0.25% Trypsin or TrypLE. Incubate 00:05:00 at RT protect from the light. 5m

4.5 Add 500 µL /well 1%-Albumin Fraction V in DPBS (-/-), collect the cells and transfer in Eppendorf tubes on ice.

4.6 Centrifuge for 00:05:00 at 400 g and 4°C. 5m

4.7 Discard supernatants and resuspend cell pellets in 250-500 µL 1%-Albumin Fraction V in DPBS (-/-), depending on the size of the cell pellet.

4.8 Filter cell suspension through Nylon filter into FC tubes.

5 Keep on ice until acquisition at the flow cytometer. Record 10,000 live events per sample.