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WORKS FOR ME 1

Fluorescently labeled polyamine uptake (via Flow Cytometry)

COMMENTS 0

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Marine Houdou[1], Peter Vangheluwe[1]

[1]KU Leuven

M Marine Houdou

ABSTRACT

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

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KEYWORDS

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GUIDELINES

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

MATERIALS TEXT

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 STARTING

- 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

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 .

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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

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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.

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