Nov 23, 2022

Evaluating endocytic rate in cells.

DOI
https://dx.doi.org/10.17504/protocols.io.8epv5jjedl1b/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.8epv5jjedl1b/v1

External link: https://www.thermofisher.com/order/catalog/product/T23366?gclid=CjwKCAjwzY2bBhB6EiwAPpUpZkuhTJcss8FLP963Hi9idgxNuMgwOubQVDObHI_Ru5cHPICunDbHdxoC24IQAvD_BwE&ef_id=CjwKCAjwzY2bBhB6EiwAPpUpZkuhTJcss8FLP963Hi9idgxNuMgwOubQVDObHI_Ru5cHPICunDbHdxoC24IQAvD_BwE:G:s&s_kwcid=AL!3652!3!447292198772!!!g!!!10506731179!109642167771&cid=bid_pca_iva_r01_co_cp1359_pjt0000_bid00000_0se_gaw_dy_pur_con

Protocol Citation: Marine Houdou, Peter Vangheluwe, Nathalie Jacobs 2022. Evaluating endocytic rate in cells.. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv5jjedl1b/v1

Manuscript citation:
Houdou M, Jacobs N, Coene J, Azfar M, Vanhoutte R, Haute CVd, Eggermont J, Daniëls V, Verhelst SHL, Vangheluwe P, Novel Green Fluorescent Polyamines to Analyze ATP13A2 and ATP13A3 Activity in the Mammalian Polyamine Transport System. Biomolecules 13(2). doi: 10.3390/biom13020337

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

Created: November 03, 2022

Last Modified: May 31, 2024

Protocol Integer ID: 72253

Keywords: ASAPCRN, endocytic rate in cell, evaluating endocytic rate, endocytic rate, flow cytometry, cytometry readout, using tagged transferrin, tagged transferrin, cell

Abstract

Assess endocytic rate in cells using tagged transferrin (Alexa647) and flow cytometry readout.

Guidelines

- Make sure to **treat** the cells in **medium without FBS**.

Materials

0.25% Trypsin-EDTA: Gibco, 25200056

Albumin Fraction V: Carl Roth, 8076.4

Alexa647-Transferrin: T23366, invitrogen

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

Endocytosis inhibitors
- Dynasore: D7693, Sigma
- Genistein: ab120112, Abcam
- Pitstop 2: SML1169, Sigma

TrypLE Express Enzyme: Gibco, 12604021

Versene Solution: Gibco, 15040

Before start

- Prepare cell culture medium without FBS and keep it at 37°C.
- 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 wellplate** that they reach **70-80% confluency** the day of the experiment.

Note

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

2 The day of the experiment, remove cell culture medium and **pre-treat** the cells in **medium without FBS** in a final volume of 500 µL /well, for 00:30:00 at 37°C, 5% CO2. The different conditions to consider are: 30m

2.1 **No** pre-treatment for:
- blank samples
- Alexa647-transferrin-only samples.

In this case, change cell culture medium to cell culture medium without FBS.

2.2 **Pre-treatment** with endocytosis inhibitors cocktail made of:
- 100 µM Dynasore,
- 50 µM Genistein and
- 50 µM Pitstop2.

2.3 After pre-treatment, keep the plate **on ice** (4°C) for 00:15:00. 15m

3 Add **Alexa647-Transferrin** at 50 µg /mL in each well, **except** for the **blank samples**.

Note

Alexa647-Transferrin is added to each well containing 500µL of either culture medium withtout FBS or pre-treated medium including endocytosis inhibitors in culture medium without FBS.

4 Incubate 00:20:00 4°C (on ice) 20m

5 Incubate 00:20:00 37°C, 5% CO2 . 20m

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

6.1 Discard medium.

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

6.3 Discard Versene or DPBS (-/-).

6.4 Add 200 µL /well 0.25 % Trypsin or TrypLE. Incubate 00:05:00 at RT . 5m

6.5 Add 500 µL /well 1 % BSA-DPBS (-/-), collect the cells and transfer in Eppendorf tubes.

6.6 Centrifuge 00:05:00 at 2500 rpm and 4°C. 5m

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

Note

Samples can't be too concentrated or too diluted to be ran on a flow cytometer.
- If samples are too concetrated, cells will form clumps, they won't be single cells anymore and this will damage the flow cytometer.
- If samples are too diluted, acquisition at the flow cytometer will take very long time.

6.8 Filter cell suspension through Nylon filter into FC tubes.

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