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WORKS FOR ME 1

Evaluating endocytic rate in cells.

COMMENTS 0

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ABSTRACT

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

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EXTERNAL LINK

https://www.thermofisher.com/order/catalog/product/T23366?gclid=CjwKCAjwzY2bBhB6EiwAPpUpZkuhTJcss8FLP963Hi9idgxNuMgwOubQVDObHI_Ru5cHPICunDbHdxoC24IQAvD_BwE&ef_id=CjwKCAjwzY2

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GUIDELINES

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

MATERIALS TEXT

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 STARTING

- 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.

30m

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:

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 .

15m

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

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.

20m

4 Incubate 00:20:00 4°C (on ice)

20m

5 Incubate 00:20:00 37°C, 5% CO2 .

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

6.1 Discard medium.

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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 5m at 2500 rpm and 4°C.

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.

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