Transferrin uptake assay to measure Clathrin-mediated endocytosis in HIPCS derived neurons.

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AUG 07, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.n2bvj38d5lk5/v1

Protocol Citation: Sakthi Kumar 2023. Transferrin uptake assay to measure Clathrin-mediated endocytosis in HIPCS derived neurons.. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvj38d5lk5/v1

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

Created: Aug 07, 2023

ABSTRACT

This protocol is used to measure Transferrin uptake as a way to investigate Clathrin-mediated endocytosis in HIPCS derived neurons, or other cell types.

Last Modified: Aug 07, 2023

PROTOCOL integer ID: 86063

Keywords: ASAPCRN, endocytosis, Clathrin, Transferrin uptake

Reagents needed:

1

1. Human Transferrin conjugated with Alexa Fluor 647 (Thermo Fisher, Catalog no: T23366).
Note: Transferrin conjugate is also available with Alexa Fluor 488, Alexa Fluor 546. The user may want to use a specific conjugate based on the need of their experiments. We have optimized this protocol for Transferrin conjugated with Alexa Fluor 647.
2. Basal Neuronal media- No added supplements like (N2, B27, NEAA, Glutamax and growth factors).
Note: The user may use their desired neuronal medium, but they have to make sure that the media does not contain any Transferrin.

Preparation of Transferrin aliquots:

2

1. Dissolve 5mg of Transferrin in 1 mL of Sterile PBS.
2. Make the aliquots of desired volume and store at 2-8 C.
3. Protect them from light.
4. Do not freeze the aliquots.
5. For long term storage, add Sodium Azide.

Experimental Protocol

1h 26m

3

1h 1m

1. Warm the basal neuronal media to 37 °C.
2. Add warmed basal media to a new 24 well plate (500uls/ well needed) in the tissue culture hood.
3. Transfer the coverslip with cells (neurons) into the well and proceed to starvation step.
4. Starvation: starve the cells by keeping the plate in the humidified incubator (37 °C, 5% CO2) up to 01:00:00
5. After 30 to 60 minutes, remove the plate from the incubator and add Transferrin (10ug/ml) to the cells and keep the plate in the incubator for 00:01:00.
6. After 1 minute, remove Transferrin and add 4% PFA for fixation.

3.1 PFA fixation:

25m

1. After adding 4% PFA wait for 00:15:00 for fixation.
2. Then wash 3 times with PBS.
3. Add Hoechst and incubate for 00:10:00.
4. Then wash 3 times with PBS and mount the coverslips on slides with Fluoromount G.
5. Image the same day or keep the slides at 4 °C if imaging the next day.

3.2 Quantification:

Use ImageJ based program to quantify Transferrin Fluorescence intensity.

Notes:

4 Cell types tested:

We have tested Transferrin uptake in multiple cell types generated from H9 Embryonic Stem Cells.

1. Cortical neurons.
2. Midbrain Dopaminergic neurons.
3. MGE derived GABA interneurons.

For the assay, we have used mature neurons from the above cell types (4 weeks after plating the progenitors).

Depending on the experiments, the user may want to test different concentrations of Transferrin and different time points of incubation. Also, the user may try pulse chase step to visualize recycling endosomes. For our experiments, we focused only on the initial uptake through Clathrin mediated endocytosis.