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

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Clusterin cellular uptake assay

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ABSTRACT

This protocol details how to efficiently monitor Clusterin and Clusterin/Substrate uptake in different cell types, like HEK293T, iNeurons and iMicroglia.

ATTACHMENTS

975-2532.docx

GUIDELINES

- To study Clusterin-A488 uptake in the presence of substrate, incubate

 [M] 1 micromolar (μM) Clusterin-A488 with the corresponding amount of substrate,
 e.g. denatured Luciferase or Aβ aggregates, in PBS for 00:20:00 at 37 °C or

 42 °C (denatured Luciferase) in a total volume of Δ 30 μL or Δ 40 μL in the case of HEK293T or iNeurons, respectively. After the incubation time dilute the mix

 1/10 in media and add it to the cells resulting in a final concentration of

 [M] 0.1 micromolar (μM) Clusterin-A488 (Δ 5 undetermined).
- To monitor substrate uptake e.g., denatured luciferase or Aβ aggregates, in the presence of Clusterin, mix [M] 0.2 micromolar (μM) of denatured Luciferase-pHrodo or [M] 0.5 micromolar (μM) of Aβ-pHrodo aggregates with the corresponding amount of unlabeled Clusterin in a total volume of [Δ] 30 μL or [Δ] 40 μL in the case of HEK293T or iNeurons, respectively. Dilute the mix 1/10 in media. pHrodo Red dye is pH sensitive dye which fluoresces brightly only in acidic environments and therefore can be used to specifically monitor phagocytosis and endocytosis, but substrates labeled with A488 can be also used.
- The indicated Clusterin-A488 or substrate concentrations and incubation times for each cell line are tentative. These parameters should be experimentally tested to be in the linear range of the assay.

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Last Modified: Feb 02, 2024 MATERIALS

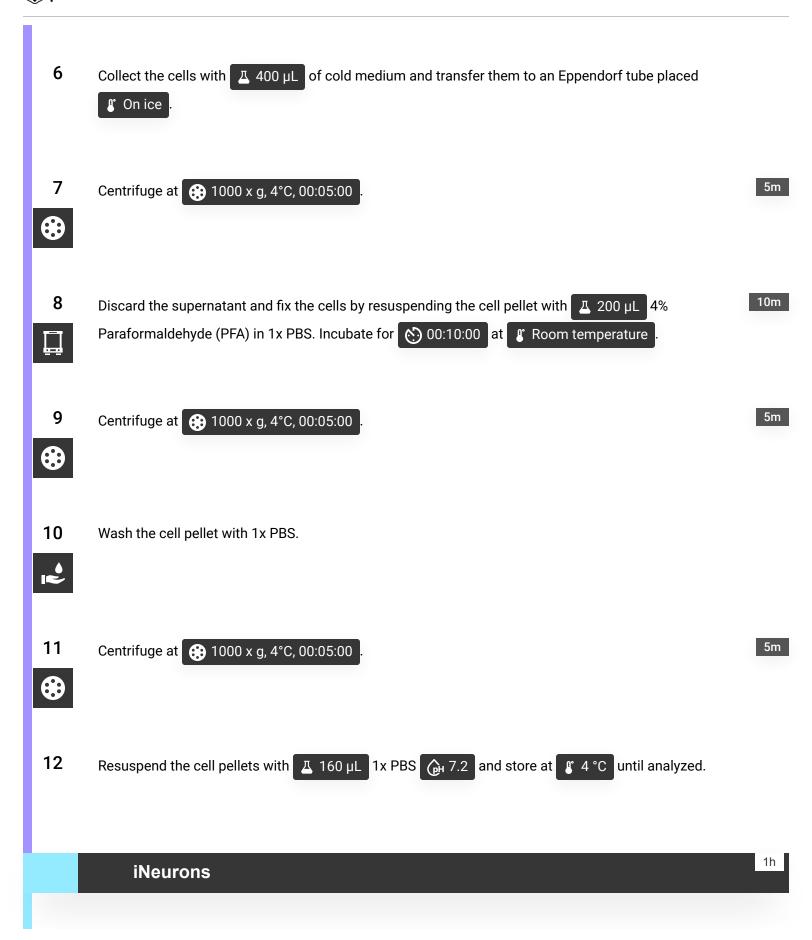
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X ACCUTASE™ 100 mL STEMCELL Technologies Inc. Catalog #7920

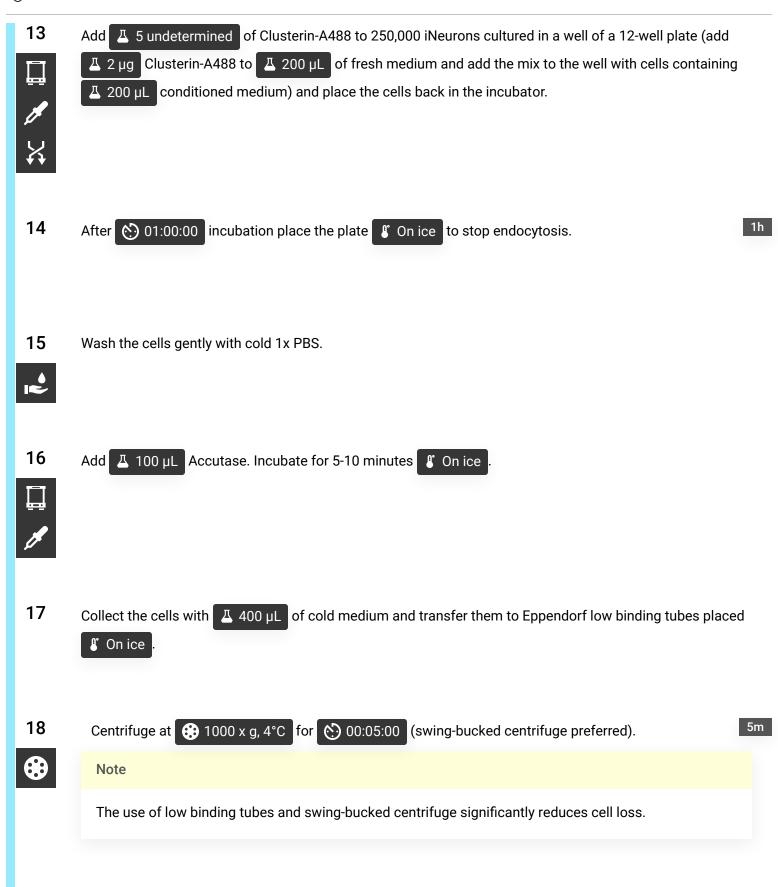
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25m Clusterin cellular uptake assay - HEK293T cells 1 Plate 100,000 HEK293T cells per well in a 24-well plate. 2 On the next day, add 🗸 5 undetermined of Clusterin-A488 together with 🗸 300 µL of fresh DMEM (without fetal bovine serum, \pm 1.5 µg in \pm 300 µL medium) to the cells and place the cells back in the incubator. 3 6) 04:00:00 incubation, place the plate on ice to stop endocytosis. 4 Wash the cells gently with cold 1x PBS. 5 Add A 100 µL TrypL Express Enzyme (Gibco). Incubate for few minutes On ice

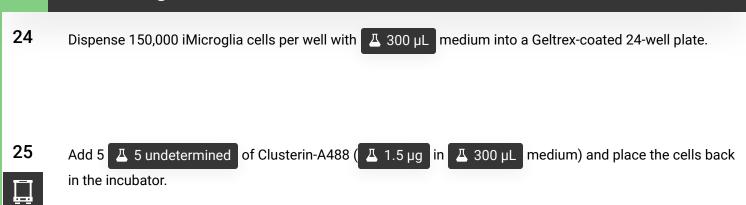


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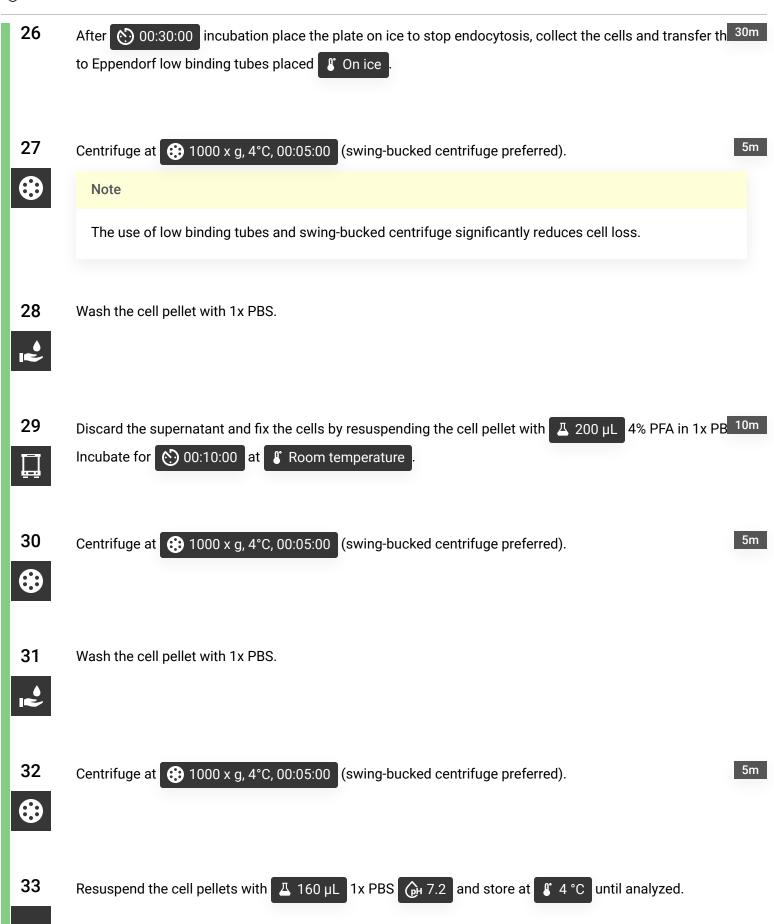


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Uptake quantification

1h 15m

34



Quantify Clusterin or substrate uptake by measuring A488 or pHrodo Red intensity inside the cells by flow cytometry. If A488 is used, add \square 50 μ L of Trypan blue solution 0.4% (refer materials section) right before measuring to quench the 488 fluorescence outside the cells.

Note

An Attune NxT flow cytometer (Thermo Fisher Scientific) can be used with the following settings:

- Alexa485: Excitation 488 nm Emission 550/30.
- pHrodo Red: Excitation 561 nm Emission 585/16.