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Lysosome proteolysis analysis with DQ-BSA

 In 1 collection

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gustavo.parfitt¹

¹Icahn School of Medicine

Ahfeldt Lab



gustavo.parfitt Parfitt

University College of London

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Protocol status: Working

We use this protocol and it's working

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Keywords: ASAPCRN, lysosomal proteolysis in astrocyte, lysosomal proteolysi, astrocyte

Abstract

Protocol to analyze lysosomal proteolysis in astrocytes generated from iPSCs.

Protocol materials

⊗ TrypLE[®]; Select Enzyme (1X), no phenol red **Thermo Fisher Catalog #12563029**

⊗ DQ[®]; Red BSA - Special Packaging **Thermo Fisher Catalog #D12051**



- 1 Coat a 96 well plate Greiner 96 well Microplates Black with gelatin 0.1% or Geltrex LDEV Free hESC Quality 5 ml Thermo Fisher Scientific Catalog #A1413302 1:100
- 2 Dissociate extracted astrocytes with 5m

 TrypLE[®]; Select Enzyme (1X), no phenol red **Thermo Fisher Catalog #12563029**

for  00:05:00 at  25 °C
- 3  300 x g, 25°C, 00:03:00 3m
- 4 Resuspend the pellet in  1 mL of astrocyte media (<https://www.sciencellonline.com/astrocyte-medium.html>).
- 5 Count cells and plate 10k cell per well in  100 µL of astrocyte media. 17h
- 6 Change the media to mature astrocyte media media in the next day. 2d
- 7 On the 3rd day, remove the media incubate with 45m

 DQ[®]; Red BSA - Special Packaging **Thermo Fisher Catalog #D12051**

diluted in mature astrocyte media for  00:45:00
- 8 Remove the media
- 9 Wash with PBS 3 times.
- 10 Add  100 µL of mature astrocyte media with vehicle or with Bafilomycin A1
 100 nanomolar (nM)



- 11 Proceed to image in the Incucyte® SX5 Live-Cell Analysis System for using the red channel

 21:00:00

21h