

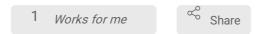


Sep 13, 2022

S Lysosome proteolysis analysis with DQ-BSA

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dx.doi.org/10.17504/protocols.io.14egn2xmyg5d/v1

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ABSTRACT

Protocol to analyze lysosomal proteolysis in astrocytes generated from iPSCs.

DOI

dx.doi.org/10.17504/protocols.io.14egn2xmyg5d/v1

PROTOCOL CITATION

gustavo.parfitt 2022. Lysosome proteolysis analysis with DQ-BSA. **protocols.io** https://protocols.io/view/lysosome-proteolysis-analysis-with-dq-bsa-cgjxtupn

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CREATED

Sep 13, 2022

LAST MODIFIED

Sep 13, 2022

PROTOCOL INTEGER ID

69975

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



5m Dissociate extracted astrocytes with **⊠**TrypLE[™] Select Enzyme (1X), no phenol red **Thermo** Fisher Catalog #12563029 for **७00:05:00** at **₹25 °C** 3m 3 300 x g, 25°C, 00:03:00 Resuspend the pellet in **1 mL** of astrocyte media (https://www.sciencellonline.com/astrocyte-medium.html). 17h 5 Count cells and plate 10k cell per well in ■100 µL of astrocyte media. 2d 6 Change the media to mature astrocyte media media in the next day. 45m On the 3rd day, remove the media incubate with **⊠**DQ[™] Red BSA - Special Packaging **Thermo** Fisher Catalog #D12051 diluted in mature astrocyte media for **© 00:45:00** Remove the media Wash with PBS 3 times. 10 Add ■100 µL of mature astrocyte media with vehicle or with Bafilomycin A1 [M]100 nanomolar (nM)

Proceed to image in the Incucyte® SX5 Live-Cell Analysis System for © 21:00:00 using the 11 red channel