



Sep 13, 2022

Lysosome proteolysis analysis with DQ-BSA

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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

- 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
 [\(https://www.sciencellonline.com/astrocyte-medium.html\)](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  **21:00:00** using the ^{21h} red channel