Oct 27, 2022

Glycolysis stress test in organoids

gustavo.parfitt[1]

[1]Icahn School of Medicine

1 *Works for me* Share

This protocol is published without a DOI.

Ahfeldt Lab

gustavo.parfitt

ABSTRACT

Glycolysis stress test in organoids

PROTOCOL CITATION

gustavo.parfitt 2022. Glycolysis stress test in organoids. **protocols.io** https://protocols.io/view/glycolysis-stress-test-in-organoids-cig6ubze

CREATED

Oct 27, 2022

LAST MODIFIED

Oct 27, 2022

PROTOCOL INTEGER ID

71934

1 Seahorse XFe96 Spheroid Microplates **Agilent Technologies Catalog ##102905-100** were coated with Laminin solution in L15+Bicarbonate: final concentration of 10 µg/mL .

2 Day 35 midbrain organoids were placed in the plates and media was changed every other day.

3 On the day of the assay
Agilent Seahorse XF Base Medium **Agilent Technologies Catalog #103334-100**
supplemented with B27 + N2 was warmed at **37 °C** and the pH as adjusted to 7.4. And 175 ul was added to the organoid plates.

4 The plate containing the organoids were warmed at **37 °C** in a non-CO2 incubator for 30 min

5 Using the
Seahorse XFp Glycolysis Stress Test **Agilent Technologies Catalog #103017-100**
kit the cartridges were loaded with loaded with Glucose (10mM), Oligomicyn (1uM) and 2-DG (50mM).

6 The run was done in

Seahorse XFe96 Analyzer
Agilent **BLE2100298**

7 After the run the organoids were lysed in RIPA buffer and total protein was quantified using BCA kit.

8 The total protein was used to normalize the ECAR and OCR.