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Glycolysis stress test in organoids

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Protocol status: Working

We use this protocol and it's working

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Abstract

Glycolysis stress test in organoids

Protocol materials

⊗ Seahorse XFe96 Spheroid Microplates **Agilent Technologies Catalog ##102905-100**

⊗ Agilent Seahorse XF Base Medium **Agilent Technologies Catalog #103334-100**

⊗ Seahorse XFp Glycolysis Stress Test **Agilent Technologies Catalog #103017-100**

- 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), Oligomycin (1uM) and 2-DG (50mM).
- 6 The run was done in

Equipment

Seahorse XFe96 Analyzer

NAME

Agilent

BRAND

BLE2100298

SKU

<https://www.agilent.com/en/product/cell-analysis/real-time-cell-metabolic-analysis/xf-analyzers/seahorse-xfe96-analyzer-740879>

LINK



- 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.