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Extracellular Oxygen Consumption Assay for mitochondrial function in cell culture

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details an Extracellular Oxygen Consumption Assay for use on dopaminergic and cortical iPSC cultures from various Parkinson's disease genetic mutations. This assay is used for the kinetic analysis of extracellular oxygen consumption rates (OCR) in real-time as a measure of the cellular respiration rate and mitochondrial function.

Attachments

PDF

Extracellular-Oxygen...

124KB

Guidelines

This protocol can be adapted for whole cell populations, isolated mitochondria, organoids, bacteria and other cultures. Please see the manufacturers Information sheet (attached) for further information.

Materials

Materials

- Extracellular Oxygen Consumption Assay (ab197243)
- 96 well culture plate

Hardware

- Nivo microplate reader (Revvity) **HH35000500**

Software

- GraphPad Prism, BOSTON, MA 02110, USA

Troubleshooting

Before start

This protocol was applied to both ventral midbrain and cortical neuronal progenitors. Extracellular oxygen consumption was measured using an oxygen-sensitive fluorescent dye (Extracellular Oxygen Consumption Assay Kit, Abcam) as per the manufacturer protocol.

Extracellular Oxygen Consumption Assay

1

1. 1 × 106 progenitor cells were seeded in each well of a 96 well plate in culture media and differentiated
as per https://dx.doi.org/10.17504/protocols.io.bu6znzf6 OR Gantner, C.W., et al., An optimized protocol for the generation of midbrain dopamine neurons under defined conditions. Star Protocols, 2020. 1(2): p.
100065.
2. On the day of analysis, the culture media was replaced with the assay media (oxygen-sensitive fluorescent dye diluted in culture media at 1:15 dilution).
3. A layer of mineral oil was then added on top of the assay media using a dropper to restrict the diffusion of oxygen.
4. The fluorescence signal was measured using a Nivo microplate reader (Revvity) at 37 °C for 02:00:00 min with readings at 00:02:00 intervals at wavelengths of 340 nm for excitation and 650nm for emission.

2h 2m

Analysis

30m

2

5. Fluorescent intensity values were normalised to the 00:30:00 time, serving as a reference point.
6. The temporal changes in fluorescence intensity were depicted using XY graphs in GraphPad Prism. To measure the rate of change, a linear segment of the graph was identified for each graph and a
simple linear regression model was fitted to this segment to calculate the slope for each PD mutation, representing the rate of change in fluorescence intensity over time. The slopes or regression coefficients of PD groups were compared with the Control using a One-Way ANOVA test, and their statistical
significance levels are marked in graphs. Experiments were repeated at least three times.

30m

Protocol references

- Gantner, C.W., et al., An optimized protocol for the generation of midbrain dopamine neurons under defined conditions. Star Protocols, 2020. 1(2): p. 100065.
- **dx.doi.org/10.17504/protocols.io.bu6znzf6**