MAR 30, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.14egn2oxyg5d/v1

Protocol Citation: michela.deleidi, María José Pérez J. 2023. Mitochondrial complex activity assays. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn2oxyg5d/v1

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

Created: Mar 28, 2023

Last Modified: Mar 30, 2023

PROTOCOL integer ID: 79527

Keywords: Mitochondrial complex activity assays, mitochondrial electron transport chain

Mitochondrial complex activity assays

In 1 collection

michela.deleidi[1], María José Pérez J.[1]

[1]German Center for Neurodegenerative Diseases (DZNE), Tübingen, 72076 Germany

Federico Bertoli

ABSTRACT

Mitochondria complex activity assays measure the activity levels of the different complexes of the mitochondrial electron transport chain (ETC).

ATTACHMENTS

676- 1425.docx

MATERIALS

Materials

- pyruvate
- malate
- ADP
- Succinate
- rotenone
- antimycin A
- TMPD (N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride, Santa Cruz Biotechnology)
- ascorbic acid
- azide
- Qproteome Mitochondrial isolation kit

MitoCheck® Complex I Activity Assay Kit **Cayman Chemical Company Catalog #700930**

Qproteome Mitochondria Isolation Kit **Qiagen Catalog #37612**

MAS buffer

	A	B
	Sucrose	70 mM
	Mannitol	220 mM
	KH_2PO_4	5 mM
	$MgCl_2$	5 mM
	EGTA	1 mM
	HEPES pH 7.4	2 mM

Mitochondrial complex activity assays

35m

1 Isolate mitochondria from HEK cells, iPSC-derived neurons, or midbrain organoids using the Qproteome Mitochondrial isolation kit (QIAGEN, Cat. No. / ID: 37612) according to manufacturer's instructions.

2 Measure Complex I (NADH oxidase/coenzyme Q reductase) using the MitoCheck Complex I Activity Assay kit (Cayman Chemical, cat# 700930).

3 Determine the rate of NADH oxidation, which is proportional to CI activity, by a decrease in absorbance at 340 nm over 00:15:00 in the presence of ubiquinone and potassium cyanide to inhibit complex IV and prevent oxidation of ubiquinone. 15m

> Note
>
> To assess CI, CII, and CIV function, we used a respirometry approach based on XFp Extracellular Flux Analysis and then proceed with steps 4-9.

4 To this end, resuspend 3 mg of purified fresh mitochondria in 200 µL of MAS buffer ([M] 70 millimolar (mM) sucrose, [M] 220 millimolar (mM) mannitol, [M] 5 millimolar (mM) KH_2PO_4, [M] 5 millimolar (mM) $MgCl_2$, [M] 1 millimolar (mM) EGTA, [M] 2 millimolar (mM) HEPES pH 7.4) and seed in XFpSeahorse microplates.

5 Centrifuge the plate at 2000 x g, 4°C, 00:05:00 . 5m

6 Measure the OCR before and after the serial addition of pyruvate + malate ([M] 5 millimolar (mM) each) + ADP 3,5 mM or 1 mM Succinate + [M] 4 micromolar (µM) rotenone, [M] 4 micromolar (µM) rotenone + [M] 8 micromolar (µM) antimycin A, 0,5 mM TMPD (N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride, Santa Cruz Biotechnology) + [M] 1 millimolar (mM) ascorbic acid, and [M] 50 millimolar (mM) azide.

7 Following each injection, record three measurements for a total period of 00:15:00 . 15m

8 Calculate Complex I-, II-, and IV-dependent respiration by subtracting OCR values from the substrates (Pyruvate + malate + ADP for CI, Succinate + rotenone for CII and TMPD + ascorbic acid for CIV) subtracted from the ones from the inhibitors (rotenone for CI, antimycin A + rotenone for CII and azide for CIV).

9 Normalize the experimental values to the protein content per well via a BCA assay.