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We use this protocol and it's working

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Mitochondrial complex activity assays

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ABSTRACT

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

ATTACHMENTS

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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.