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Mitochondrial complex activity assays

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Protocol status: Working

We use this protocol and it's working

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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



[676- 1425.docx](#)

13KB

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 ₂ PO ₄	5 mM
	MgCl ₂	5 mM
	EGTA	1 mM
	HEPES pH 7.4	2 mM

Troubleshooting

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 μ L of MAS buffer (70 millimolar (mM) sucrose, 220 millimolar (mM) mannitol, 5 millimolar (mM) KH_2PO_4 , 5 millimolar (mM) MgCl_2 , 1 millimolar (mM) EGTA, 2 millimolar (mM) HEPES 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 (5 millimolar (mM) each) + ADP 3,5 mM or 1 mM Succinate + 4 micromolar (μ M) rotenone, 4 micromolar (μ M) rotenone + 8 micromolar (μ M) antimycin A, 0,5 mM TMPD (N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride, Santa Cruz Biotechnology) + 1 millimolar (mM) ascorbic acid, and 50 millimolar (mM) azide.
- 7 Following each injection, record three measurements for a total period of 00:15:00 .
- 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 +



15m



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.