
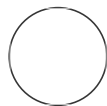




MAR 30, 2023

## 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

[676- 1425.docx](#)

### OPEN ACCESS

#### DOI:

[dx.doi.org/10.17504/protocols.io.14egn2oxyg5d/v1](https://dx.doi.org/10.17504/protocols.io.14egn2oxyg5d/v1)

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

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**PROTOCOL integer ID:**  
79527

**Keywords:** Mitochondrial complex activity assays, mitochondrial electron transport chain

## 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**

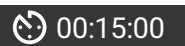
### MAS buffer

A	B
Sucrose	70 mM
Mannitol	220 mM
KH <sub>2</sub> PO <sub>4</sub>	5 mM
MgCl <sub>2</sub>	5 mM
EGTA	1 mM
HEPES pH 7.4	2 mM

## Mitochondrial complex activity assays

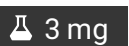



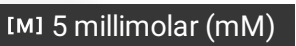
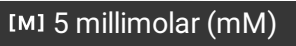
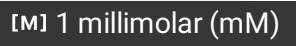
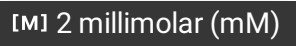

35m

- 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

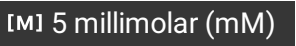

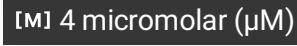
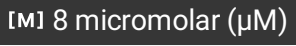
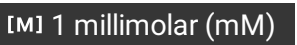


Note

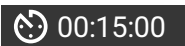
To assess CI, CII, and CIV function, we used a respirometry approach based on XFp Extracellular Flux Analysis and then proceed with steps 4-9.

- 4 To this end, resuspend  3 mg of purified fresh mitochondria in  200  $\mu$ L of MAS buffer (  70 millimolar (mM) sucrose,  220 millimolar (mM) mannitol,  5 millimolar (mM)  $\text{KH}_2\text{PO}_4$ ,  5 millimolar (mM)  $\text{MgCl}_2$ ,  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 ( $\mu$ M) rotenone,  4 micromolar ( $\mu$ M) rotenone +  8 micromolar ( $\mu$ 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 . 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.

