



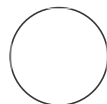
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# Complex I activity assay

In 6 collections

María José Pérez J.<sup>1</sup>, michela.deleidi<sup>1</sup>

<sup>1</sup>German Center for Neurodegenerative Diseases (DZNE), Tübingen, 72076 Germany



Hariam Raji

## ABSTRACT

This protocol describes the complex I activity assay.

## ATTACHMENTS

[404-877.docx](#)


## GUIDELINES

**Reference for analysis:** <https://www.nature.com/articles/nprot.2012.058>

## MATERIALS

### KIT:

Mitocheck Complex I activity assay kit

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

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

## OPEN ACCESS

**DOI:**  
[dx.doi.org/10.17504/protocols.io.36wgq7ok5vk5/v1](https://dx.doi.org/10.17504/protocols.io.36wgq7ok5vk5/v1)

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

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**PROTOCOL integer ID:**  
61490

**Keywords:** Complex I activity assay, - Extinction coefficient, Enzyme activity

## BEFORE START INSTRUCTIONS


- All assays are carried out at 25 °C .
- After mitochondrial isolation (Qproteome Mitochondria Isolation Kit. QIAGEN Cat. No. / ID: 37612), resuspend the final pellet in 50 µL of storage buffer, keep isolated mitochondria On ice .
- In a ventilated hood, weigh out 6.5 mg of KCN and dissolve in 1 mL of 0.1 Molarity (M) NaOH (stock solution of 100 millimolar (mM) )
- Label two polystyrene tubes as A and B. For 20 reactions prepare:

A	B
Tube A (1 ml)	Tube B (675 µl)
910 µl of Complex I activity buffer	625 µl of Complex I activity buffer
20 µl of 100mM KCN (1 mM)	30 µl of NADH assay reagent
50 µl FF-BSA Assay Reagent	20 µl of Ubiquinone assay reagent
20 µl of Vehicle	



## Protocol

5m

- 1 Distribute the contents of tube A and B in strips suitable for multichannel use.
- 2 In a Half Volume 96-well clear plate add 50 µL of the contents of tube A to each well.
- 3 Add 20 µL of sample to each well.

- 4 Place plate in plate reader and add  30  $\mu\text{L}$  of B to each well.



- 5 Immediately measure absorbance at 340 nm in kinetic read mode (30 seconds intervals for  00:05:00 at  25  $^{\circ}\text{C}$  )

5m

## Calculations

- 6 The specific activity of complex I is calculated as  $\text{nmol min}^{-1} \text{mg}^{-1}$  of protein according to the following equation:

Enzyme activity ( $\text{nmol min}^{-1} \text{mg}^{-1}$ ) =  $(\Delta \text{Absorbance}/\text{min} \times 1,000)/[(\text{extinction coefficient} \times \text{volume of sample used in ml}) \times (\text{sample protein concentration in } \text{mg ml}^{-1})]$ .

- 7 Extinction coefficient for NADH  $6.2 \text{ mM}^{-1} \text{ cm}^{-1}$ .