



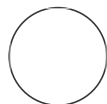
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# Complex I activity assay

In 1 collection

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

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

## 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**



## BEFORE START INSTRUCTIONS


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

A	B
Tube A (1 ml)	Tube B (675 $\mu\text{L}$ )
910 $\mu\text{L}$ of Complex I activity buffer	625 $\mu\text{L}$ of Complex I activity buffer
20 $\mu\text{L}$ of 100mM KCN (1 mM)	30 $\mu\text{L}$ of NADH assay reagent
50 $\mu\text{L}$ FF-BSA Assay Reagent	20 $\mu\text{L}$ of Ubiquinone assay reagent
20 $\mu\text{L}$ 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  $50\ \mu\text{L}$  of the contents of tube A to each well.  

- 3 Add  $20\ \mu\text{L}$  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  $6.2 \text{ mM}^{-1} \text{ cm}^{-1}$ .