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Complex I activity assay

In 2 collections

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Maria Jose Perez J.[1], michela.deleidi[1]

[1]German Center for Neurodegenerative Diseases (DZNE), Tübingen, 72076 Germany

Federico Bertoli
ASAP

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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, ASAPCRN, assay, complex, activity, protocol

Abstract

This protocol describes the complex I activity assay.

Attachments

404-877.docx
15KB

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

- 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 [M] 0.1 Mass Percent NaOH (stock solution of [M] 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 µL 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 °C)

5m

Calculations

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

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

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