**Complex I activity assay**

KIT: Mitocheck Complex I activity assay kit. Cayman Item No: 700930

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

Before starting

* 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 50ul 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 M NaOH (stock solution of 100mM)
* Label two polystyrene tubes as A and B. For 20 reactions prepare:

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

* Distribute the contents of tube A and B in strips suitable for multichannel use.
* In a Half Volume 96-well clear plate add 50 l of the contents of tube A to each well.
* Add 20 l of sample to each well.
* Place plate in plate reader and add 30 l of B to each well.
* Immediately measure absorbance at 340 nm in kinetic read mode (30 seconds intervals for 5 minutes at 25°C)

Calculations

* 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)].

* Extinction coefficient for NADH 6.2 mM-1 cm-1