A convenient, easy-to-follow shortened protocol is provided with this assay.
For a detailed protocol go to www.caymanchem.com/pdfs/700930.pdf

MitoCheck® Complex I Activity Assay Kit Short Protocol

Item No. 700930

REAGENT PREPARATION

1. **Mitochondrial Complex I Activity Assay Buffer - (Item No. 700931)** - Ready to use as supplied; warm to room temperature and vortex prior to use.

2. **NADH Assay Reagent - (Item No. 700932)** - Dissolve in 155 µl of UltraPure water; stable on ice for three hours; store at -20°C for up to 2 weeks.

3. **Mitochondrial Inhibitors - (Not Supplied)**
   KCN-Use extreme care when preparing the KCN reagent. Weigh 6.5 mg KCN in a ventilated hood; dissolve in 1 ml of 0.1 M NaOH to make a 500 mM stock solution; store on ice; make fresh 3 hrs before use.
   Rotenone-To ensure inhibition of complex I, use concentrations ≥1 µM; can be made up in DMSO or ethanol; if making up in DMSO, avoid freeze/thaws. Use appropriate PPE.
   Antimycin A-To ensure inhibition of complex III, use concentrations ≥10 µM; can be made up in DMSO or ethanol. Use appropriate PPE.

4. **Reaction Stock Solutions -** Add the following reagents into 2 polystyrene tubes (sufficient for 20 reactions).

Tube A (1 mL)	Tube B (675 mL)
910 µl of Complex I Activity Assay Buffer	625 µl of Complex I Activity Assay Buffer
20 µl of 100 mM KCN (1 mM)	30 µl of NADH Assay Reagent
50 µl of FF-BSA Assay Reagent	20 µl of Ubiquinone Assay Reagent
20 µl Bovine Heart Mitochondria Assay Reagent*	

*Isolated mitochondria can settle over time; mix well before use.

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PERFORMING THE ASSAY

1. Add 50 µl of the contents of tube A to each well.
2. Add 20 µl of test compounds, positive control, or vehicle diluted in Assay Buffer to the appropriate wells. Allow for pre-incubation if required.
3. Add 30 µl of the contents of tube B to each well to start the reaction.
4. Immediately place plate on plate reader and measure absorbance at 340 nm (30 second intervals for 15 min @ 25°C).

CALCULATIONS

1. Plot time-dependent reaction data as absorbance (y-axis) *versus* time (x-axis).
2. To determine the reaction rate, calculate the slope for the linear portion of the curve.
3. Determine % activity using the equation below.
4. To generate a concentration response curve, plot the % activity as a function of test compound concentration.

$$\text{Complex I Activity (\%)} = \left[\frac{\text{Rate of Sample wells}}{\text{Rate of Vehicle Control}}\right] \times 100$$