A convenient, easy-to-follow shortened protocol is provided with this assay. For a detailed protocol go to www.caymanchem.com/pdfs/700950.pdf

#### MitoCheck<sup>®</sup> Complex II/III Activity Assay Kit Short Protocol Item No. 700950

#### **REAGENT PREPARATION**

- 1. Mitochondrial Complex III Activity Assay Buffer (Item No. 700951) Ready to use as supplied; warm to room temperature and vortex prior to use.
- 2. 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.

<u>Rotenone</u>-To ensure inhibition of complex I, use concentrations  $\geq 1 \mu$ M; can be made up in DMSO or ethanol; if making up in DMSO, avoid freeze/thaws. Use appropriate PPE.

<u>Antimycin A</u>-To ensure inhibition of complex III, use concentrations  $\geq 10 \ \mu$ M; can be made up in DMSO or ethanol. Use appropriate PPE.

<u>2-Thenoyltrifluoroacetone (TTFA)</u>-To ensure inhibition of complex II, use concentrations  $\geq 1$  mM; can be made up in DMSO or ethanol. Use appropriate PPE.

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

Tube A (1 mL)	Tube B (675 μL)
958 µl of Complex III Activity Assay Buffer	607 µl of Complex III Activity Assay Buffer
20 µl Bovine Heart Mitochondria Assay Reagent*	8 μl of Succinate Assay Reagent
2 μl of 1 mM Rotenone	60 µl of Cytochrome C Assay Reagent
20 μl of 100 mM KCN (1 mM)	

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



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## **PERFORMING THE ASSAY**

- 1. Add 50  $\mu$ l of the contents of tube A to each well.
- 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  $\mu l$  of the contents of tube B to each well to start the reaction.
- Immediately place plate in plate reader and measure absorbance at 550 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.

Complex II/III Activity (%) = 
$$\begin{bmatrix} Rate of Sample wells \\ Rate of Vehicle Control \end{bmatrix} x 100$$



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07/10/2015