A convenient, easy-to-follow shortened protocol is provided with this assay.
For a detailed protocol go to www.caymanchem.com/pdfs/701000.pdf

MitoCheck® Complex V Activity Assay Kit Short Protocol

Item No. 701000

REAGENT PREPARATION

1. **Mitochondrial Complex V Activity Assay Buffer - (Item No. 701001)** - Ready to use as supplied; warm to room temperature and vortex prior to use.

2. **Mitochondrial Complex V Enzyme Mix - (Item No. 701002)** - Ready to use as supplied; thaw on ice prior to use.

3. **Bovine Heart Mitochondria Assay Reagent - (Item No. 700019)** - Ready to use as supplied; thaw on ice prior to use.

4. **Mitochondrial Complex V NADH Reagent - (Item No. 701003)** - Reconstitute vial with 120 µl UltraPure water prior to use.

5. **Mitochondrial Complex V NADH Reagent - (Item No. 701003)** - Reconstitute vial with 120 µl UltraPure water prior to use

6. **Mitochondrial Inhibitors - (Not Supplied)**
   Oligomycin-Use stock at 1 mg/ml; 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.

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

Tube A (1 mL)	Tube B (675 mL)
978 µl of Complex V Activity Assay Buffer	635 µl of Complex V Assay Enzyme Mix
20 µl Bovine Heart Mitochondria Assay Reagent*	20 µl of Complex V ATP Reagent
20 µl Rotenone - not supplied (from 1 mM stock)	20 µl of Complex V NADH 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 positive control or test compounds. 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 30 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.

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