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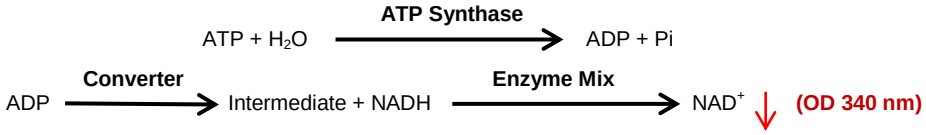
Mitochondrial ATP synthase Activity Assay Kit (Colorimetric)

rev 11/21

(Catalog # K2112-100; 100 assays; Store at -80 °C)

I. Introduction:

Mitochondrial ATP synthase (EC 3.6.3.14), also known as mitochondrial complex V or F_1F_0-ATP synthase is a multi-subunit membrane-bound enzyme of mitochondria that utilizes the proton electrochemical gradient generated by the respiratory chain for ATP synthesis. It consists of two domains, an inner membrane embedded domain (F_0) and a membrane extrinsic domain (F_1), which is also the catalytic core of the enzyme. In addition to its critical role in ATP synthesis, ATP synthase regulates the flux of oxidative phosphorylation, mitochondrial signaling by reactive oxygen species, cell death etc. Defects in ATP synthase have been associated with human diseases including neuromuscular disorders, heart failure etc. Thus, ATP synthase may be used as a therapeutic drug target in the treatment of many diseases. **BioVision's Mitochondrial ATP Synthase Activity Assay Kit** is a simple, plate-based colorimetric assay for measuring ATP synthase in isolated mitochondrial samples. In this assay, ATP Synthase hydrolyzes ATP to ADP. In the subsequent steps, ADP together with ATP synthase converter and enzyme mix oxidizes NADH to NAD^+, which is monitored via a decrease in absorbance at 340 nm. A specific ATP synthase inhibitor, oligomycin is also included in the kit. Sample specific ATP synthase activity is obtained by measuring the sample ATP synthase activity in the presence and absence of oligomycin. The assay provides a simple, rapid, and sensitive method to measure ATP synthase activity in isolated mitochondria.



II. Application:

- Measurement of Mitochondrial ATP synthase enzymatic activity in isolated mitochondria

III. Sample Type:

- Isolated mitochondria

IV. Kit Contents:

Components	K2112-100	Cap Code	Part Number
ATP Synthase Assay Buffer	25 ml	WM	K2112-100-1
ATP	2 vials	Orange	K2112-100-2
ATP Synthase Converter	1 vial	Purple	K2112-100-3
NADH	1 vial	Yellow	K2112-100-4
ATP Synthase Enzyme Mix	1 vial	Red	K2112-100-5
Oligomycin	25 µl	Amber	K2112-100-6
Purified Mitochondria	8 µl	Green	K2112-100-7
96-well Half Area Clear Plate	--	--	K2112-100-8

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer capable of reading absorbance in kinetic mode
- Deionized water

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20 °C except the Purified Mitochondria, which should be stored at -80 °C. Briefly centrifuge all small vials before opening. Read the entire protocol before performing the assay.

- **ATP Synthase Assay Buffer:** Ready to use. Store at 4 °C or -20 °C. Warm to room temperature (RT) before use.
- **ATP:** Reconstitute one vial in 55 µl dH_2O to prepare the ATP stock solution. Pipette up and down to dissolve completely. Each vial can be used to carry out up to 54 reactions. Store at -20 °C. Keep on ice while in use.
- **ATP Synthase Converter:** Reconstitute the vial in 220 µl ATP Synthase Assay Buffer. Divide into aliquots and store at -20 °C. Keep on ice while in use.
- **NADH:** Reconstitute the vial with 120 µl ATP Synthase Assay Buffer to prepare the NADH stock solution. Centrifuge briefly after mixing. Store at -20 °C, protected from light. Keep on ice while in use.
- **ATP Synthase Enzyme Mix:** Reconstitute the vial in 120 µl ATP Synthase Assay Buffer. Divide into aliquots and store at -20 °C. Keep on ice while in use.
- **Oligomycin (in DMSO):** Ready to use. Bring to RT before use, to dissolve the DMSO. Store at -20 °C.
- **Purified Mitochondria:** Ready to use. **Store at -80 °C**. Avoid multiple freeze-thaw cycles. Keep on ice while in use. **Note:** Please keep on dry ice if not using immediately.
- **96-well Half Area Clear Plate:** Store the plate at RT or 4 °C.

VII. Mitochondrial ATP Synthase Assay Protocol:

1. **Sample Preparation:** Isolate mitochondria from cultured cells or tissue using any preferred procedures. We recommend using Mitochondria Isolation Kit for Tissue & Cultured Cells (BioVision Cat# K288-50) for maximum yield and consistency. Estimate the protein concentration of isolation mitochondrial samples using BCA Protein Assay Kit (BioVision Cat# K818). **Isolated mitochondria should undergo a minimal of 3 freeze-thaw cycles (freeze in a dry ice/ethanol bath and then thaw at RT or 37 °C) and placed on ice during the assay.** Prepare isolated mitochondria to working concentration (0.2-1.0 mg/ml) in ATP Synthase Assay Buffer. Sample dilutions should be prepared immediately before performing the assay.
   Notes:
   - We recommend testing several dilutions of the mitochondrial samples to make sure that the activity falls in the linear range of the assay.

- Isolated mitochondria should be stored at -80 °C, unless being used immediately.

2. **Assay Preparation:** Prepare 1:20 dilution of Oligomycin by mixing 2 µl Oligomycin stock with 38 µl ATP Synthase Assay Buffer. Prepare 1:10 dilution of Purified Mitochondria by mixing 1 µl Purified Mitochondria with 9 µl ATP Synthase Assay Buffer. Prepare wells of the 96-well Half Area Clear Plate labeled as **Background Control [BC], Sample [S], Sample with Oligomycin [SC], Purified Mitochondria,** and **Purified Mitochondria with Oligomycin** as shown below.

	Background Control [BC]	Sample [S]	Sample with Oligomycin [SC]	Purified Mitochondria	Purified Mitochondria with Oligomycin
Diluted Oligomycin	--	--	2 µl	--	2 µl
Test Sample	--	1-5 µl (1 - 5 µg protein)	1-5 µl (1 - 5 µg protein)	--	--
Diluted Purified Mitochondria	--	--	--	2 µl	2 µl
ATP Synthase Assay Buffer	80 µl	to 80 µl	to 80 µl	78 µl	76 µl

Incubate the plate at 30 °C for 10-15 min, protected from light.

3. **Reaction Mix Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare 20 µl Reaction Mix containing:

	Reaction Mix
ATP Synthase Assay Buffer	15 µl
ATP Synthase Converter	2 µl
ATP	1 µl
NADH	1 µl
ATP Synthase Enzyme Mix	1 µl

Mix well and add 20 µl of the Reaction Mix to all wells including [BC], [S], [SC], Purified Mitochondria and Purified Mitochondria with Oligomycin. **Note:** Prepare Reaction Mix immediately, before adding to the wells.

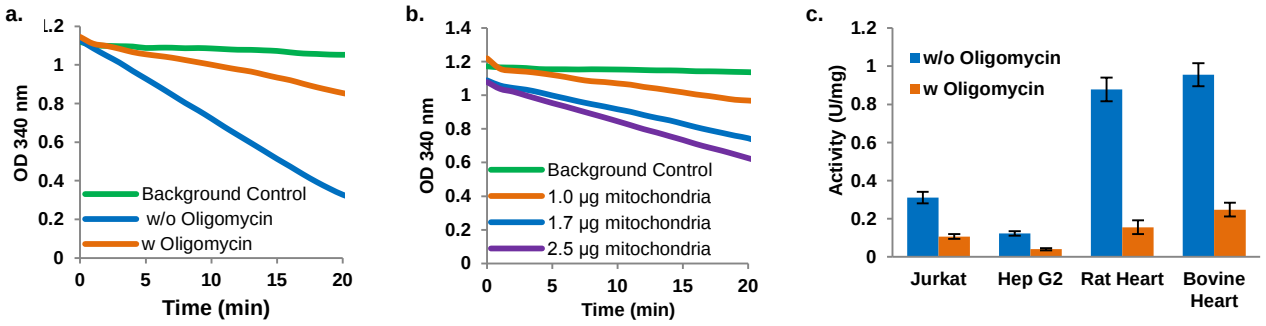
4. **Measurement:** Immediately measure the absorbance of all wells at 340 nm in kinetic mode at 30 °C for 5-30 min. For Sample [S], Sample with Oligomycin [SC] and Background Control [BC], choose any two time points (t_1 & t_2) in the linear range of the curve and obtain the corresponding absorbance (OD_1 and OD_2).

5. **Calculation:** Calculate the net absorbance in **Sample [S]: ($\Delta OD_S = OD_1 - OD_2$); Sample with Oligomycin [SC]: ($\Delta OD_{SC} = OD_1 - OD_2$)** and **Background Control [BC]: ($\Delta OD_{BC} = OD_1 - OD_2$)** during the reaction time ($\Delta t = t_2 - t_1$). Subtract the ΔOD_{BC} readings from ΔOD_S to get the **corrected Sample readings, ($\Delta OD_{Sample\ Corrected} = \Delta OD_S - \Delta OD_{BC}$)**. Subtract the ΔOD_{BC} readings from ΔOD_{SC} to get the **corrected Sample with Oligomycin, ($\Delta OD_{Sample\ with\ Oligomycin\ Corrected} = OD_{SC} - \Delta OD_{BC}$)**. Calculate the sample ATP Synthase activity using the following equation:

$$\text{Sample ATP Synthase Activity} = \frac{\left(\frac{\Delta OD_{"sample\ corrected"}}{\Delta t} - \frac{\Delta OD_{"sample\ with\ Oligomycin\ corrected"}}{\Delta t}\right) x\ (0.1)\ \text{x}\ \text{D}}{6.22\ x\ 0.56\ x\ \text{V}\ \text{x}\ \text{P}} = \text{Units/mg}$$

Where:
0.1 = Reaction volume (ml)
6.22 = Millimolar extinction coefficient of NADH ($mM^{-1}cm^{-1}$)
0.56 = Light path for included half-area microplate (cm)
V = Volume added into the reaction well (ml)
P = Sample protein concentration (mg/ml)
D = Sample Dilution Factor (D = 1 for undiluted samples)

Unit Definition: One unit of ATP Synthase activity is the amount of enzyme that converts 1µmol of NADH per min under the assay conditions at 30 ºC.



Figures. (a) ATP Synthase activity in purified mitochondria with and without Oligomycin. **(b)** ATP Synthase activity using varying concentrations of Jurkat cell mitochondria (isolated using BioVision Cat# K288-50). **(c)** ATP Synthase activity in mitochondria isolated from Jurkat cells, HepG2 cells, rat heart (isolated using BioVision Cat# K288-50) and bovine heart mitochondria (obtained commercially) with and without Oligomycin. **Note:** *Net ATP Synthase activity in sample = Activity in reaction without Oligomycin – Activity in reaction with Oligomycin.* All assays were performed following kit protocol.

VIII. Related Products:

Mitochondrial Complex I Activity Colorimetric Assay Kit (K968)
Mammalian Mitochondrial Isolation Kit for Tissue & Cultured Cells (K288)
BCA Protein Assay Kit (K813-2500)
Mitochondrial Complex III Activity Assay Kit (K520)
Yeast Mitochondria Isolation Kit (K259)
Dounce Tissue Homogenizer (1998-1)

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