

# ATPase Activity Assay Kit (Colorimetric)

rev. 8/19

(Catalog # K417-100; 100 assays; Store at Multiple Temperatures)

## I. Introduction:

ATPase (Adenosine Triphosphatase: EC 3.6.1.3) is an important enzyme for maintaining the cell membrane potential, transporting ions and regulating cellular volume. It catalyzes the decomposition of ATP into ADP and a free phosphate ion. The hydrolysis of ATP is highly exergonic releasing energy that is utilized in several cellular processes. There are many classes of ATPases including  $\text{Na}^+/\text{K}^+$ -ATPase,  $\text{H}^+/\text{K}^+$ -ATPase,  $\text{Ca}^{2+}$ -ATPase, etc. The deficiency of mitochondrial ATPase is serious: for example,  $\text{Na}^+/\text{K}^+$ -ATPase deficiency increases anxiety-related behavior, while  $\text{Ca}^{2+}$ -ATPase deficiency leads to exertional muscle pain syndrome. Therefore, accurate detection of ATPase activity is valuable for the diagnosis and mechanistic studies of some of these diseases. BioVision's ATPase Activity Assay kit provides a quick and easy method for monitoring ATPase activity in various samples. In the assay, ATPase hydrolyzes ATP releasing ADP and a free phosphate ion, and through linked reactions, a strong, stable chromophore is generated (OD 650 nm). The assay is simple, sensitive, high-throughput adaptable and can detect ATPase Activity less than 0.005 U/L.



## II. Application:

- Measurement of ATPase activity in various tissues/cells
- Analysis of energy-generating pathways
- Analysis of  $\text{Na}^+/\text{K}^+$ -ATPase mediated signal transduction pathways, e.g. MAPK, ROS etc.

## III. Sample Type:

- Animal tissues: Liver, heart, kidney, etc.
- Cell culture: Adherent or Suspension Cells

## IV. Kit Contents:

Components	K417-100	Cap Code	Part Number
ATPase Assay Buffer	25 ml	WM	K417-100-1
ATPase Substrate	2 vials	Blue	K417-100-2
ATPase Developer	3 ml	Clear	K417-100-3
Phosphate Standard (10 mM)	0.5 ml	Yellow	K417-100-4
ATPase Positive Control	1 vial	Orange	K417-100-5

## V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (plate reader)

## VI. Storage, Handling and Reagent Preparation:

Store the kit at multiple temperatures. Centrifuge vials briefly, prior to use.

- **ATPase Assay Buffer:** Warm to room temperature (RT) before use. Store at 4°C.
- **ATPase Substrate:** Reconstitute each vial with 110  $\mu\text{l}$  dH<sub>2</sub>O. Pipette up and down to dissolve. Aliquot and store at -20°C. Use within two months.
- **ATPase Developer:** Ready to use as supplied. Store at RT.
- **Phosphate Standard (10 mM):** Warm to room temperature (RT) before use. Store at RT.
- **ATPase Positive Control:** Reconstitute with 100  $\mu\text{l}$  ATPase Assay Buffer and mix thoroughly. Keep on ice while in use. Aliquot and store at -20°C. Use within two months.

## VII. ATPase Activity Assay Protocol:

**1. Sample Preparation:** For whole cells or tissue lysate, rapidly homogenize tissue (40 mg) or cells ( $2 \times 10^6$ ) with 400  $\mu\text{l}$  ice cold ATPase Assay Buffer, and place sample on ice for 10 min. Centrifuge at 10,000 x g at 4°C for 10 min and collect the supernatant. *Important: The phosphate in tissue samples and cell lysates will interfere with assay.* Remove endogenous phosphate by using ammonium sulfate method: Aliquot the tissue samples (100  $\mu\text{l}$ ) to a clean centrifuge tube, add saturated ammonium sulfate (BioVision Cat. # 7096) to a final concentration of 3.2 M and place on ice for 20 mins. Spin down samples at 10,000 g at 4°C for 10 min, discard the supernatant, and resuspend the pellet back to the original volume. Add Samples (2-20  $\mu\text{l}$ ) in duplicates onto a clear 96-well plate labeled as Background Control, and Sample. Adjust final volume to 100  $\mu\text{l}$  with ATPase Assay Buffer. **For Reagent Control:** Add 100  $\mu\text{l}$  ATPase Assay Buffer. **For ATPase Positive Control:** Dilute 10  $\mu\text{l}$  of ATPase Positive Control into 190  $\mu\text{l}$  of ATPase Assay Buffer. Add 2-20  $\mu\text{l}$  of ATPase Positive Control into wells and adjust final volume to 100  $\mu\text{l}$  with ATPase Assay Buffer.

### Note:

- For Unknown Samples, we suggest testing several volumes to ensure the readings are within the Standard Curve range.
- Many detergents commonly found in laboratories contain high amounts of phosphates which can adhere to clean glassware. It is highly recommended to use **disposable plastic labware** for all Samples, Standards and Reagents to avoid contamination.

- 2. Phosphate Standard Curve:** Dilute 10  $\mu$ l of the 10 mM Phosphate Standard into 990  $\mu$ l dH<sub>2</sub>O, mix well to generate 100  $\mu$ M working Phosphate Standard. Add 0, 10, 20, 30, 40 and 50  $\mu$ l of 100  $\mu$ M Phosphate Standard to individual wells to generate 0, 1, 2, 3, 4 and 5 nmol/well of Phosphate Standard. Adjust volume to 200  $\mu$ l/well with ATPase Assay Buffer.
- 3. Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 100  $\mu$ l Reaction Mix containing:

	<u>Reaction Mix</u>	<u>Background Control Mix*</u>
ATPase Assay Buffer	98 $\mu$ l	100 $\mu$ l
ATPase Substrate	2 $\mu$ l	----

Add 100  $\mu$ l of the Reaction Mix to each well containing the Positive Control, Reagent Control\*\* and Samples. Incubate at 25°C for 30 min. **Do not add Reaction Mix to the Standards.**

**Notes:**

\*For Sample Background Control, add 100  $\mu$ l of Background Control mix to each well and mix well.

\*\* For Reagent Control, prepare a well adding 100  $\mu$ l ATPase Assay Buffer (see step 1).

- 4. Measurement:** Add 30  $\mu$ l ATPase Assay Developer to all Standard, ATPase Positive Control, Sample(s), Sample Background Control and Reagent Control wells. Incubate at 25°C for 30 min & measure OD at 650 nm in Endpoint mode (at the end of the incubation time).
- 5. Calculation:** Subtract the 0 Standard reading from all Standard readings. Plot the Phosphate Standard Curve. Correct the Sample Background by subtracting the higher value derived from the Background Control or Reagent Control from all Sample readings (*Experimental results indicated that Reagent Background Control shows higher absorbance values*). Calculate the ATPase activity of the Sample:  $\Delta OD = A_2 - A_1$ . Apply the  $\Delta OD$  to the Phosphate Standard Curve to get B nmol of phosphate generated by ATPase during the reaction time (t = 30 min).

$$\text{Sample ATPase Activity} = \frac{B}{(t \times V)} \times D = \text{nmol/min}/\mu\text{l} = \text{mU}/\mu\text{l} = \text{U/ml}$$

Where: **B** is the Phosphate amount from Standard Curve (nmol)

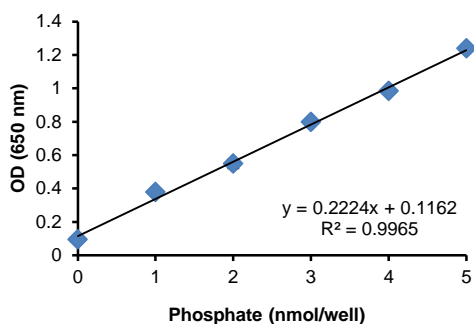
**t** is the reaction time (min)

**V** is the Sample volume added into the reaction well ( $\mu$ l)

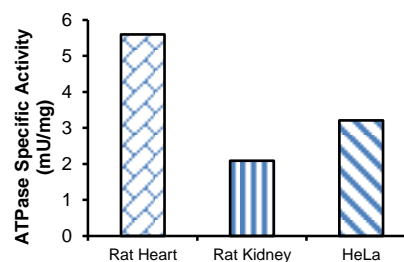
**D** is the Sample dilution factor

Unit Definition: One unit of ATPase is the amount of enzyme that will generate 1.0  $\mu$ mol of phosphate per min at pH 7.5 at 25°C.

(a)



(b)



**Figures: A)** Phosphate Standard Curve. **B)** Specific ATPase Activity were calculated in lysates prepared from Rat Heart (35  $\mu$ g), Rat Kidney (15  $\mu$ g), and HeLa Cell Lysate (5.4  $\mu$ g). Assays were performed following kit protocol.

**VIII. Related Products:**

Phosphate Assay Kit (K410)

Pyruvate Colorimetric /Fluorometric Assay Kit (K609)

Pyruvate Dehydrogenase Activity Assay Kit (K679)

Glucose-6-Phosphate Dehydrogenase Assay Kit (K757)

Phosphoglucomutase Assay Kit (K774)

Glucose Dehydrogenase Activity Assay Kit (K786)

Glucose Uptake Colorimetric Assay (K676)

Glucose Uptake Fluorometric Assay (K666)

Glucose-6-Phosphate Assay Kit (657)

Hexokinase Assay Kit (K789)

Phosphoglucose Isomerase Assay Kit (K775)

Glucose-1-Phosphate Assay Kit (K697)

**FOR RESEARCH USE ONLY! Not to be used on humans.**