



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 Na⁺/K⁺-ATPase, H⁺/K⁺-ATPase, Ca²⁺-ATPase, etc. The deficiency of mitochondrial ATPase is serious: for example, Na⁺/K⁺-ATPase deficiency increases anxiety-related behavior, while Ca²⁺-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 Na⁺/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 µl dH₂O. 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 µl 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 (2×10^6) with 400 µl 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 µl) 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 µl) in duplicates onto a clear 96-well plate labeled as Background Control, and Sample. Adjust final volume to 100 µl with ATPase Assay Buffer. **For Reagent Control:** Add 100 µl ATPase Assay Buffer. **For ATPase Positive Control:** Dilute 10 µl of ATPase Positive Control into 190 µl of ATPase Assay Buffer. Add 2-20 µl of ATPase Positive Control into wells and adjust final volume to 100 µl 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.

