PRODUCT INFORMATION & MANUAL

ATPase Activity Activity Assay Kit (Colorimetric)

NBP2-59733

For research use only.

Not for diagnostic or therapeutic procedures.

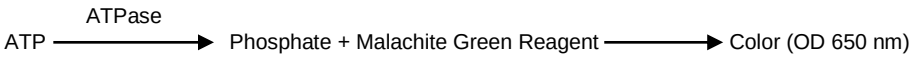
www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

ATPase Activity Assay Kit (Colorimetric)

(Catalog #NBP2-59733 Store at -20°C)

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, Ca^{2+} -ATPase, etc. The deficiency of mitochondrial ATPase is serious: for example, Na^+/K^+-ATPase deficiency increases anxiety-related behavior, while 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. Novus'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	NBP2-59733	Cap Code
ATPase Assay Buffer	25 ml	WM
ATPase Substrate	2 vials	Blue
ATPase Developer	3 ml	Clear
Phosphate Standard (10 mM)	0.5 ml	Yellow
ATPase Positive Control	1 vial	Orange

V. User Supplied Reagents and Equipment:

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

VI. Storage, Handling and Reagent Preparation:

Store kit at -20ºC, protected from light. Centrifuge vials briefly, prior to use.

- **ATPase Assay Buffer:** Warm to room temperature before use. Store at 4ºC.
- **ATPase Substrate:** Reconstitute one well with 110 µl dH_2O. Pipette up and down to dissolve. Aliquot and store at -20°C. Use within two months.
- **ATPase Developer:** Ready to use as supplied and keep it at room temperature.
- **ATPase Positive Control:** Reconstitute with 100 µl 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 minutes. 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 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 mins, discard the supernatant, and resuspend the pellet back to the original volume. Add samples (2-20 µl) in duplicates onto a clean 96-well plate (labeled "background control", and sample ATPase activity"). For reagent control: add 100 µl ATPase Assay Buffer. Adjust final volume to 100 µl with 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.

2. **Phosphate Standard Curve:** Dilute 10 µl of the 10 mM Phosphate Standard into 990 µl dH_2O, mix well to generate 100 µM working Phosphate Standard. Add 0, 10, 20, 30, 40 and 50 µl of 100 µM Phosphate Standard to individual wells to generate 0, 1, 2, 3, 4 and 5 nmol/well of Phosphate Standard. Adjust volume to 200 µl/well with ATPase Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 100 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix*
ATPase Assay Buffer	98 µl	100 µl
ATPase Substrate	2 µl	----

Add 100 µl of the Reaction Mix to each well containing the Positive Control, Reagent Control** and test samples. Incubate at 25°C for 30 min. ***Do not add Reaction Mix to the Standards.***

Note:

*For sample background control, add 100 µl of Background Control mix to each well and mix well.

** For Reagent Control, prepare a well adding 100 µl ATPase Assay Buffer (see step 1).

4. **Measurement:** Add 30 µl ATPase Assay Developer to all standards, ATPase Positive Control and Test Samples and Sample Background Controls. Incubate at 25°C for 30 min and measure OD at 650 nm in Endpoint mode (i.e., at the end of incubation time).
5. **Calculation:** Subtract the 0 standard reading from all standard readings. Plot the phosphate standard curve. Correct 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 test sample: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the Phosphate standard curve to get B nmol of phosphate generated by ATPase during the reaction time (e.g. t = 30 min).

Sample ATPase Activity = B/(tX V) x D = nmol/min/µl = mU/µl = 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 (µl)
D is the sample dilution factor

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

(a) (b)

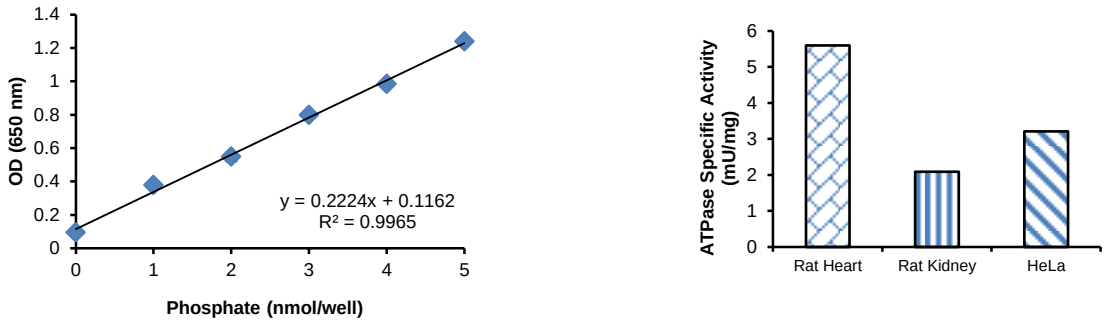


Figure: **A)** Phosphate Standard Curve. **B)** Specific ATPase Activity were calculated in lysates prepared from Rat Heart (35 µg), Rat Kidney (15 µg), and Hela Cell Lysate (5.4 µg). Assays were performed following kit protocol.

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