ELISA PRODUCT INFORMATION & MANUAL

ATPase Activity Activity Assay Kit (Colorimetric)

NBP2-59733

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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1. Overview

ATPase Activity Activity Assay Kit (Colorimetric) (NBP2-59733) 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.

Prepare all samples, controls and standards.

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

Add ATPase Assay Developer to all standards, ATPase Positive Control, Test Samples and Sample Background Controls. Incubate at 25°C for 30 minutes.

Measure OD at 650 nm in Endpoint mode.

2. Materials Supplied and Storage

Store kit except for the ATPase Developer at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

The ATPase developer contains Malachite green so it is normal to occasionally see small amounts of green precipitate. This will not affect the performance of the assay. You can centrifuge and take the supernatant to avoid taking the precipitate.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
ATPase Assay Buffer	25 mL	-20°C	4°C
ATPase Substrate	2 vials	-20°C	-20°C
ATPase Developer	3 mL	RT	RT
Phosphate Standard (10 mM)	0.5 mL	-20°C	Room Temp
ATPase Positive Control	1 vial	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

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

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 ATPase Assay Buffer

Warm to room temperature before use. Store at 4°C.

5.2 Phosphate Substrate

Warm to room temperature before use. Store at 4°C.

5.3 ATPase Substrate

Reconstitute one vial with 110 µL dH_2O. Pipette up and down to dissolve. Aliquot and store at -20°C. Use within two months.

5.4 ATPase Developer

Ready to use as supplied. Store at room temperature.

5.5 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.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Dilute 10 µL of the 10 mM Phosphate Standard into 990 µL dH_2O, mix well to generate 100 µM working Phosphate Standard.
2. 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.
3. Adjust volume to 200 µL/well with ATPase Assay Buffer.

Δ Note: 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.

7. Sample Preparation

1. For whole cells or tissue lysate, rapidly homogenize tissue (40 mg) or cells (2 x 10^6) with 400 µL ice cold ATPase Assay Buffer, and place sample on ice for 10 minutes.
2. Centrifuge at 10,000 x g at 4°C for 10 minutes and collect the supernatant.
3. 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 (such as ab273568) 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.
4. Add samples (2-20 µL) in duplicates onto a clear 96-well plate (labeled "background control", and sample ATPase activity"). Adjust final volume to 100 µL with ATPase Assay Buffer.

   Δ Note: For unknown samples, we suggest testing several volumes to ensure the readings are within the standard curve range.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Reagent Control and ATPase Positive Control:

1. For Reagent control: add 100 µL ATPase Assay Buffer.
2. 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.

8.2 Reaction Mix:

1. Prepare 100 µL of Reaction Mix and Background Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
ATPase Assay Buffer	98	100
ATPase Substrate	2	-

2. Add 100 µL of Reaction Mix into each well containing the Positive Control, Reagent Control and test samples. Do not add Reaction Mix to the Standards.
3. Incubate at 25°C for 30 minutes.

   ∆ **Note**: For sample background control, add 100 µL of Background Control mix to each well and mix well.

8.3 Measurement:

1. Add 30 µL ATPase Assay Developer to all standards, ATPase Positive Control, Test Samples, Sample Background Controls and Reagent control wells.
2. Incubate at 25°C for 30 min and measure OD at 650 nm in Endpoint mode (ie. at the end of incubation time).

9. Data Analysis

1. Subtract the 0 standard reading from all standard readings.
2. Plot the phosphate standard curve.
3. 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).
4. Calculate the ATPase activity of the Sample: ΔOD = A2 – A1 where A2 is the raw sample absorbance and A1 is the higher value derived from the Background Control or Reagent Control for all Sample readings (Experimental results indicated that Reagent Background Control shows higher absorbance values).
5. 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).

$$Sample\ ATPase\ Activity = \frac{B}{(tX\ V)} * D = = \ nmol/min/\mu L\ = \ mU/\mu L\ = \ U/mL$$

Where:

B = amount of Phosphate in the sample well calculated from standard curve in [nmol].

V = sample volume added in the sample wells [µL].

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

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.

10. Typical Data

Data provided **for demonstration purposes only**.

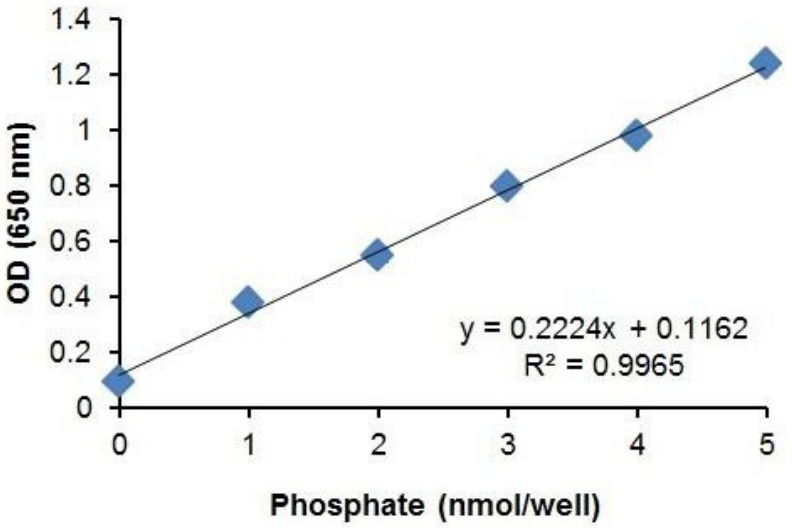

Figure 1. Phosphate Standard Curve.

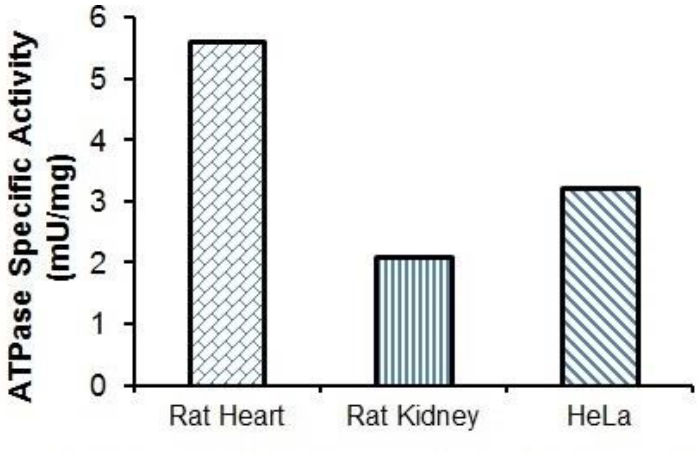

Figure 2. Specific ATPase Activity were calculated in lysates prepared from Rat Heart (35 µg), Rat Kidney (15 µg), and HeLa Cell Lysate (5.4 µg). Assays were performed following kit protocol.

11. Notes