



**PRODUCT INFORMATION &
MANUAL**

**ATPase Activity Assay Kit
(Colorimetric)
*NBP3-25849***

For research use only.
Not for diagnostic or therapeutic
procedures.

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Novus kits are guaranteed for 6 months from date of receipt

ATPase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25849

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Measuring instrument: Spectrophotometer


Sensitivity: 0.05U/mL

Detection range: 0.05-30U/mL

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help.

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.



Application

This kit can be used to measure the ATPase activity in samples, such as cell membranes, mitochondria, microsomes samples.

Detection significance

ATPase exists on cell membrane and organelle membrane. It is a protease in biomembrane. It plays an important role in material transport, energy conversion and information transmission.

Detection principle

ATPase can decompose ATP to produce ADP and inorganic phosphorus. The activity of ATPase can be calculated indirectly by measuring the content of inorganic phosphorus.

Kit components

Item	Specification	Storage
Reagent 1	Liquid, 10 mL × 3 vials	2-8°C
Reagent 2	Liquid, 10 mL × 1 vial	2-8°C
Reagent 3	Liquid, 10 mL × 1 vial	2-8°C
Reagent 4	Powder, 5 vials	-20°C
Preparation of reagent 4 solution: dissolve a vial of powder with 5 mL of double distilled water. Prepare fresh solution before use. The unused solution can be stored at -20°C for 1 week.		
Reagent 5	Powder, 2 vials	2-8°C
Preparation of reagent 5 solution: dissolve a vial of powder with 5 mL of double distilled water before use. It can be properly heated to dissolve.		
Reagent 6	Powder, 1 vial	RT
Preparation of reagent 6 solution: dissolve a vial of powder with 10 mL of double distilled water before use. It can be store at room temperature. [Note]: Reagent 6 is a oversaturated solution, so it is recommended to fully dissolve it with 90~100°C 10.3 mL (expand with heat and contract with cold phenomenon) of double distilled water and stir with glass rod. The unused Reagent 6 may crystallize, it must be dissolved before use.		
Reagent 7	Liquid, 10 mL × 1 vial	2-8°C
Preparation of reagent 7 solution: dilute the reagent 7 with double distilled water to a final volume of 25 mL before use. It can be store at 2-8°C.		
Reagent 8	Powder, 5 vials	2-8°C, shading light
Preparation of reagent 8 solution: dissolve a vial of powder with 40 mL of double distilled water before use. It can be store at 2-8°C with shading light for a week.		
Reagent 9	Powder, 2 vials	RT
Preparation of reagent 9 solution: dissolve a vial of powder with 100 mL of double distilled water before use. It can be store at room temperature.		
Reagent 10	2.5 mol/L H ₂ SO ₄ , 100 mL × 2 vials	2-8°C

Reagent 11	10 mol/L Phosphorus Standard Stock Solution, 10 mL × 1 vial	2-8°C
Preparation of 1 μmol/mL Phosphorus standard application solution: dilute the reagent 11 with double distilled water for 10 times.		
Preparation of Phosphorus determination solution: mix the double distilled water, 2.5 mol/L H ₂ SO ₄ , reagent 8 and reagent 9 at a ratio of double distilled water: 2.5 mol/L H ₂ SO ₄ : Reagent 8: Reagent 9 = 2:1:1:1. The prepared Phosphorus determination solution should be light-yellow. Colorless means the reagent is invalid, and blue means the reagent has been contaminated. This reagent should be prepared fresh before use.		

Experimental instruments

Tube, Micropipettor, Vortex mixer, 37°C thermostat water bath, Spectrophotometer (660 nm)

Operation steps

1. Enzymatic reaction

	Tube A	Tube B	Tube C	Tube D	Tube E
Reagent 1 (μL)	70	70	50	70	50
Reagent 2 (μL)	20	20	20		20
Reagent 3 (μL)				20	20
Reagent 4 (μL)	20	20	20	20	20
Reagent 5 (μL)			20	20	20
Reagent 6 (μL)	20	20	20		
Sample (μL)		100	100	100	100
Mix fully and react accurately in 37°C water bath for 10 min.					
Reagent 7 (μL)	50	50	50	50	50
Sample (μL)	100				
Mix fully and centrifuge at 3000~4000 rpm for 10 min, then take 100 μL supernatant for phosphorus determination.					

2. Phosphorus determination

	Standard tube	Tube A	Tube B	Tube C	Tube D	Tube E
1 $\mu\text{mol/mL}$ Phosphorus standard application solution (μL)	100					
Supernatant (μL)		100	100	100	100	100
Phosphorus determination solution (μL)	2000	2000	2000	2000	2000	2000

Mix fully and incubate in 45°C water bath for 20 min, then cool down to room temperature. Set the spectrophotometer to zero with double-distilled water and measure the OD value of each tube at 660 nm with 1 cm diameter cuvette.

Note: 1. Tube A is contrast tube; Tube B is Na^+/k^+ -ATPase tube; Tube C is Mg^{2+} -ATPase tube. Tube D is Ca^{2+} -ATPase tube; Tube E is $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase tube. (Please choose corresponding tube number to do experiment according to requirement.).

2. Reference sampling:

Erythrocyte membrane: 100 μL , 2% tissue cell membrane: 100 μL ,
2% mitochondria: 100 μL , 2% micro mitochondrion: 100-200 μL

Calculation of results

Definition: The enzyme amount that 1 μmol inorganic phosphorus produced by the decomposition of ATP per mg protein per hour is defined as 1 unit.

ATPase Activity (U/mgprot)

$$= \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\text{OD}_{\text{Standard}}} \times \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}} \times \text{Concentration of standard (1 } \mu\text{mol/mL)}$$

$$\times \text{Dilution factor of sample in reaction system} \times 6^* \div \text{Protein concentration of sample (mgprot/mL)}$$

6*: the reaction time is 10 min, but it is 1 hour in definition.

Reference value

	Na ⁺ /K ⁺ -ATPase (U/mgprot)	Mg ²⁺ -ATPase (U/mgprot)	Ca ²⁺ -ATPase (U/mgprot)
2% Rat cardiac muscle homogenate	8.5±0.78	4.82±0.62	5.32±0.62
10% Rat kidney homogenate	1.09±0.19	0.81±0.17	1.03±0.18
10% Rat liver homogenate	0.76±0.13	0.80±0.16	0.79±0.21

Notes

1. This kit is for research use only.
 2. Please progress strictly with operation procedures.
 3. Do not use components from different batches of kit.
 4. The valid period of kit is 12 months and the expiration date is on the packing box.
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