H+K+-ATPase Activity Assay Kit

Catalog No: E-BC-K122-S
Method: Colorimetric method
Specification: 100 Assays (Can detect 50 samples without duplication)
Measuring instrument: Spectrophotometer

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.

Phone: 240-252-7368(USA)       Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.
Application
The kit is used for the determination of H⁺K⁺-ATPase activity in animal tissue, cultured cells samples.

Detection significance
H⁺K⁺-ATPase is a member of the P-type ATPase family and mediates the exchange and transport of intracellular hydrogen ions and extracellular potassium ions. Gastric H⁺K⁺-ATPase (HKAg) mainly exists in gastric mucosal wall cells and a small amount in renal medulla. As a membrane-bound protein, HKAg has the function of acidifying gastric contents and activating pepsin, and can be used as a therapeutic target for peptic ulcer disease. However, colonic HKA (HKAc) exists in the colon or other tissues and mediates the reabsorption of active K.

Detection principle
ATPase can decompose ATP to produce ADP and inorganic phosphorus. The activity of ATPase can be expressed by measuring the production amount of inorganic phosphorus in unit time. The inorganic phosphorus reacts with ammonium molybdate in acidic solution to form ammonium molybdate compound, which is reduced with reducing agent to form molybdenum blue, and has absorption peak at 660 nm. Determine the concentration of molybdenum blue to calculate the amount of inorganic phosphorus.

Kit components

<table>
<thead>
<tr>
<th>Item</th>
<th>Component</th>
<th>Specification</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>Buffer Solution</td>
<td>20 mL × 1 vial</td>
<td>2-8°C, 3 months</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>Accelerator</td>
<td>8 mL × 1 vial</td>
<td>2-8°C, 3 months</td>
</tr>
<tr>
<td>Reagent 3</td>
<td>Acid Solution</td>
<td>8 mL × 1 vial</td>
<td>2-8°C, 3 months</td>
</tr>
<tr>
<td>Reagent 4</td>
<td>Substrate</td>
<td>Powder × 2 vials</td>
<td>-20°C, 3 months</td>
</tr>
</tbody>
</table>

Preparation of reagent 4 application solution: dissolve a vial of powder with 5 mL double distilled water. The prepared solution can be stored at -20°C for a week.

| Reagent 5 | Inhibitor          | Powder × 2 vials    | 2-8°C, 3 months  |

Preparation of reagent 5 application solution: dissolve a vial of powder with 5 mL double distilled water and heat appropriately. The prepared solution can be stored at -20°C for a week.

| Reagent 6 | Complexing Agent   | 6 mL × 1 vial       | 2-8°C, 3 months  |
| Reagent 7 | Stop Solution      | 10 mL × 1 vial      | 2-8°C, 3 months  |

Preparation of reagent 7 application solution: Dilute reagent 7 with double distilled water to the final volume of 25 mL before use. The prepared solution can be stored at 2-8°C for 3 months.

| Reagent 8 | Reducing Agent     | Powder × 2 vials    | 2-8°C, 3 months, shading light |

Preparation of reagent 8 application solution: Dissolve 1 vial of powder with 30 mL of double distilled water before use. The prepared solution can be stored at 2-8°C with shading light for a week.

| Reagent 9 | Chromogenic Agent  | Powder × 1 vial     | 2-8°C, 3 months  |

Preparation of reagent 9 application solution: Dissolve 1 vial of powder with 60 mL of double distilled water.
distilled water before use. The prepared solution can be stored at 2-8°C for 3 months. If there is a small amount of insoluble powder, take supernatant directly, it will not affect the results.

<table>
<thead>
<tr>
<th>Reagent 10</th>
<th>2.5 mol/L Sulphuric Acid</th>
<th>60 mL × 1 vials</th>
<th>2-8°C, 3 months</th>
</tr>
</thead>
</table>

**Preparation of phosphorus assay reagent:** mix double distilled water, reagent 10, reagent 8 application solution, reagent 9 application solution at a ratio of 2:1:1:1. Prepared solution should be pale yellow. If it is colorless or blue, it should be invalid or phosphorus pollution. Prepare the fresh Phosphorus assay reagent before use.

<table>
<thead>
<tr>
<th>Reagent 11</th>
<th>Standard Stock Solution</th>
<th>10 mL × 1 vial</th>
<th>2-8°C, 3 months</th>
</tr>
</thead>
</table>

**Preparation of 0.5 μmol/mL standard:** dilute the reagent 11 with double distilled water for 20 times. The prepared solution can be stored at 2-8°C with for a week.

**Experimental instrument**
Test tube, micropipettor, vortex mixer, incubator, centrifuge, spectrophotometer (660nm).

**Sample pretreatment**
*Sample requirements: The samples should be detect within 24 hours after collecting.*

1. **Tissue sample:**
   Take 0.02-1 g tissue sample, wash with normal saline (0.9% NaCl) at 2-8°C. Absorb the water with filter paper and weigh. Then add 9 times the volume of normal saline according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S).

2. **Cell sample:**
   Collect the cells with cell scraper (Don’t use trypsin or EDTA). Add normal saline (0.9% NaCl) at a ratio of cell number (10⁶): normal saline (μL) =1: 300-500, then treat the sample with mechanical homogenate or sonication on ice. Centrifuge at 4°C at 10000 g for 10 min and collect the supernatant for measurement. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S).
Operation steps

1. **Preparation of working solution A and B:**
   Prepare needed amount of the fresh **working solution A** (for control tube) and **working solution B** (for sample tube) according to the following table.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Working solution A</th>
<th>Working solution B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1 (μL)</td>
<td>130 × (n+2*)</td>
<td>130 × (n+2*)</td>
</tr>
<tr>
<td>Reagent 2 (μL)</td>
<td></td>
<td>80 × (n+2*)</td>
</tr>
<tr>
<td>Reagent 3 (μL)</td>
<td>120 × (n+2*)</td>
<td></td>
</tr>
<tr>
<td>Reagent 4 application solution (μL)</td>
<td>40 × (n+2*)</td>
<td>40 × (n+2*)</td>
</tr>
<tr>
<td>Reagent 5 application solution (μL)</td>
<td>40 × (n+2*)</td>
<td>40 × (n+2*)</td>
</tr>
<tr>
<td>Reagent 6 (μL)</td>
<td></td>
<td>40 × (n+2*)</td>
</tr>
<tr>
<td>Total amount of mixture reagent (μL)</td>
<td>330 × (n+2*)</td>
<td>330 × (n+2*)</td>
</tr>
</tbody>
</table>

   [Note] n refers to the number of sample.

2*: Prepare 2 more tubes of working solution A and working solution B, respectively.

2. **Operation procedure**

1) **Enzymatic reaction**
   (1) Control tube: take 330 μL of working solution A to 1.5 mL EP tube.
   Sample tube: take 330 μL of working solution B to 1.5 mL EP tube.
   (2) Add 100 μL of sample to **sample tube**.
   (3) Mix fully and incubate at 37°C for 10 min.
   (4) Add 50 μL of reagent 7 application solution to each tube.
   (5) Add 100 μL of sample to **control tube**.
   (6) Mix fully and centrifuge at 2000 g for 10 min, take 400 μL supernatant of each tube for phosphorus assay.

2) **Phosphorus assay**
   (1) Standard tube: take 400 μL of 0.5 μmol/mL standard to 5 mL EP tube
   Control tube: take 400 μL of supernatant from corresponding sample tube to 5 mL EP tube.
   Sample tube: take 400 μL of supernatant from corresponding sample tube to 5 mL EP tube.
   (2) Add 2000 μL of phosphorus assay reagent to each tube.
   (3) Mix fully, incubate at 45°C for 10 min and cool to room temperature.
   (4) Set the spectrophotometer to zero with distilled water and measure the OD of each tube at 660 nm with 1 cm optical path quartz cuvette.
Note: It can be refer to the following operating table.

1) Enzymatic reaction

<table>
<thead>
<tr>
<th>Tube number</th>
<th>Control tube</th>
<th>Sample tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working solution A (μL)</td>
<td>330</td>
<td></td>
</tr>
<tr>
<td>Working solution B (μL)</td>
<td></td>
<td>330</td>
</tr>
<tr>
<td>Sample (μL)</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

Mix fully and incubate at 37°C for 10 min.

| Reagent 7 application solution (μL) | 50 | 50 |
| Sample (μL)                         | 100|

Mix fully and centrifuge at 2000 g for 10 min, take 400 μL supernatant of each tube for phosphorus assay.

2) Phosphorus assay

<table>
<thead>
<tr>
<th>Tube number</th>
<th>Standard tube</th>
<th>Control tube</th>
<th>Sample tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 μmol/mL standard (μL)</td>
<td>400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant of control tube (μL)</td>
<td></td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>Supernatant of sample tube (μL)</td>
<td></td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>Phosphorus assay reagent (μL)</td>
<td>2000</td>
<td>2000</td>
<td>2000</td>
</tr>
</tbody>
</table>

Mix fully, incubate at 45°C for 10 min and cool to room temperature. Set the spectrophotometer to zero with distilled water and measure the OD of each tube at 660 nm with 1 cm optical path quartz cuvette.
Calculation of results

1. Unit definition:
   1 μmol of inorganic phosphorus produced by the decomposition of ATP by ATPase of 1 mg of tissue protein per hour is defined as 1 ATPase activity unit.

2. Formula:

   \[
   \text{H}^+\text{K}^+\text{-ATPase activity} = \frac{A_2 - A_1}{A_3} \times C \times 4.8* \times 6^{**} \times C_{pr} \times f
   \]

   [Note]:
   
   $A_1$: the OD value of control
   $A_2$: the OD value of sample
   $A_3$: the OD value of standard
   $C$: the concentration of standard, 0.5 μmol/mL
   $4.8^*$: the dilution factor of the sample in the reaction system
   $6^{**}$: the reaction time is 10 min, but the time in unit definition is an hour.
   $C_{pr}$: Concentration of protein in sample, mgprot/mL
   $f$: Dilution factor of sample before tested.

Technical parameter

1. The intra-assay CV is 4.4% and the inter-assay CV is 9.8%.

2. The recovery of the kit is 109%.

Notes

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.
5. The tubes used in assay must be disposed strictly without a trace of phosphorus. It is better to use disposable tubes or new tubes to avoid pollution of phosphorus which is the key for success.
6. All the containers of reagents should be dedicated, including the pipette of drawing sulfuric acid and distilled water containers.
7. The protein concentration of the sample to be tested should be less than 3 mg/mL.