Pyruvate Assay Kit

Catalog No: E-BC-K130-S
Method: Colorimetric method
Specification: 100 Assays

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

If you have any problems, 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**

This kit can be used to measure pyruvate content of serum, plasma and tissue samples. This kit (100 Assays) can detect 96 samples.

**Detection significance**

Pyruvic acid is the simplest of the alpha-keto acids, with a carboxylic acid and a ketone functional group. Pyruvic acid can be made from glucose through glycolysis, converted back to carbohydrates (such as glucose) via gluconeogenesis, or to fatty acids through a reaction with acetyl-CoA. It can also be used to construct the amino acid alanine and can be converted into ethanol or lactic acid via fermentation. Pyruvic acid supplies energy to cells through the citric acid cycle (also known as the Krebs cycle) when oxygen is present (aerobic respiration), and alternatively ferments to produce lactate when oxygen is lacking (lactic acid fermentation).

**Detection principle**

Pyruvic acid can react with chromogenic agent and the reaction product is reddish brown in alkaline solution. The depth of color is directly proportional to the pyruvate content. The pyruvate content can be calculated by measuring the OD value at 505 nm.

**Experimental instruments**

Test tube, Micropipettor, Vortex mixer, Incubator, Spectrophotometer (505 nm)

**Kit components**

<table>
<thead>
<tr>
<th>Component</th>
<th>Specification</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>Clarificant</td>
<td>12 mL × 1 vial</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>Chromogenic agent</td>
<td>60 mL × 1 vial</td>
</tr>
<tr>
<td>Reagent 3</td>
<td>Alkaline solution</td>
<td>50 mL × 6 vials</td>
</tr>
<tr>
<td>Reagent 4</td>
<td>2 μmol/mL Sodium pyruvate standard</td>
<td>1.5 mL × 1 vial</td>
</tr>
</tbody>
</table>

**Preparation of 0.2 μmol/mL Sodium pyruvate standard solution:** Dillute Reagent 4 with double distilled water for 10 times.
**Operation procedures**

It is recommended to take 2~3 samples which expected large difference to do pre-experiment before formal experiment.

1. **For serum (plasma) sample:** Detect the sample directly.
   Note: Hemolysis and turbid sample may affect the result. If there are turbidity or flocculent precipitates in the samples, centrifuge the sample and then take the supernatant for detection. The serum can be store at 4°C for 3 days or -20°C for more than 15 days.

**Operation steps**

a. **Blank tube:** Add 0.1 mL of double-distilled water and 0.5 mL of Reagent 2.
   
   **Standard tube:** Add 0.1 mL of 0.2 μmol/mL Sodium pyruvate standard solution and 0.5 mL of Reagent 2.
   
   **Sample tube:** Add 0.1 mL of Sample and 0.5 mL of Reagent 2.

b. Mix fully with vortex mixer for 5 sec, then incubate the tubes at 37°C for 10 min.

c. Add 2.5 mL of Reagent 3 into each tube. Mix fully with vortex mixer for 5 sec, then incubate the tubes at room temperature for 5 min.

d. Set to zero with double-distilled water and measure the OD value of each tube at 505 nm with 1 cm optical path cuvette.

**Note:** It can be refer to the following operating table

<table>
<thead>
<tr>
<th></th>
<th>Blank tube</th>
<th>Standard tube</th>
<th>Sample tube</th>
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<tbody>
<tr>
<td>Double-distilled water (mL)</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 μmol/mL Sodium pyruvate standard solution (mL)</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (mL)</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Reagent 2 (mL)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mix fully, incubate at 37°C for 10 min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent 3 (mL)</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Mix fully with vortex mixer for 5 sec, then incubate the tubes at room temperature for 5 min. Set to zero with double-distilled water and measure the OD value of each tube at 505 nm with 1 cm optical path cuvette.
2. **For tissue sample**

Weight the sample accurately and homogenized in normal saline on ice, the volume of normal saline (mL): the weight of the tissue (g) =9:1. Centrifuge the tissue homogenate at 3500 rpm for 10 min to prepare 10% tissue homogenate and carry out the assay (The supernatant after centrifugation must be clarified, and if there is turbidity, it must be centrifuged again). Meanwhile, determine the concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S).

**Operation steps**

a. **Blank tube:** Add 0.1 mL of double-distilled water, 0.1 mL of Reagent 1 and 0.5 mL of Reagent 2.

   **Standard tube:** Add 0.1 mL of 0.2 μmol/mL Sodium pyruvate standard solution, 0.1 mL of Reagent 1 and 0.5 mL of Reagent 2.

   **Sample tube:** Add 0.1 mL of sample, 0.1 mL of Reagent 1 and 0.5 mL of Reagent 2.

b. Mix fully with vortex mixer for 5 sec, then incubate the tubes at 37°C for 10 min.

b. Add 2.5 mL of Reagent 3 into each tube. Mix fully with vortex mixer for 5 sec, then incubate the tubes at room temperature for 5 min.

d. Measure the OD value of each tube at 505 nm (1 cm optical path cuvette, set to zero with double-distilled water.

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<tr>
<td>0.2 μmol/mL Sodium pyruvate standard solution (mL)</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>10% Tissue homogenate (mL)</td>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Reagent 1 (mL)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Reagent 2 (mL)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
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Mix fully, incubate at 37°C for 10 min.

| Reagent 3 (mL) | 2.5 | 2.5 | 2.5 |

Mix fully with vortex mixer for 5 sec, then incubate the tubes at room temperature for 5 min. Set to zero with double-distilled water and measure the OD value of each tube at 505 nm with 1 cm optical path cuvette.
Calculation of results

1. For serum (plasma) sample
   Pyruvate content (μmol/mL)
   \[ \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}} \times \text{Concentration of standard (0.2 μmol/mL)} \]

2. For tissue sample
   Pyruvate content (μmol/mgprot)
   \[ \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}} \times \text{Concentration of standard (0.2 μmol/mL)} \div \text{Protein concentration of sample (mgprot/mL)} \]

Technical parameters

1. The sensitivity of the kit is 0.006 μmol/mL.
2. The intra-assay CV is 1.32 % and the inter-assay CV is 1.49 %.
3. The recovery of the kit is 100.18 %.
4. The linear range of the kit is 0.006-2.0 μmol/mL.

Notes

1. This product is for scientific research use 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.