Alanine Aminotransferase (ALT/GPT) Assay Kit

Catalog No: E-BC-K200
Method: Colorimetric method
Specification: 100 Assays

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
This kit can be used to measure ALT/GPT activity in animal serum (plasma), animal tissue, culture cells and cell culture supernatant, etc. This kit (100 Assays) can detect 48 samples.

Detection significance
Alanine aminotransferase (ALT) is widely found in plasma and various tissues of the body, including liver, kidney, heart and skeletal muscle. ALT is an important pyridoxal phosphate dependent enzyme in the intermediate metabolism of glucose and protein. Clinically, the activity of serum alanine aminotransferase is often used as a marker for alcoholic liver disease, liver cirrhosis and acute viral hepatitis.

Detection principle
ALT catalyze the amino conversion reaction between alanine and α-ketoglutaric acid to produce pyruvic acid and glutamic acid at pH 7.4 and 37°C. Then phenylhydrazine was added to form phenylhydrazone with pyruvic acid. Phenylhydrazone is reddish brown under alkaline conditions. ALT activity can be calculated by measuring the OD values at 505 nm.

Kit components

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Components</th>
<th>Specifications</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Buffer Solution</td>
<td>2 mL × 1 vial</td>
<td>4°C, 6 months</td>
</tr>
<tr>
<td>2</td>
<td>2 μmol/mL Sodium pyruvate standard solution</td>
<td>2 mL × 1 vial</td>
<td>4°C, 6 months</td>
</tr>
<tr>
<td>3</td>
<td>Substrate solution</td>
<td>60 mL × 1 vial</td>
<td>4°C, 6 months, shading light</td>
</tr>
<tr>
<td>4</td>
<td>Chromogenic agent</td>
<td>60 mL × 1 vial</td>
<td>4°C, 6 months, shading light</td>
</tr>
<tr>
<td>5</td>
<td>Aqueous alkali</td>
<td>60 mL × 1 vial</td>
<td>4°C, 6 months</td>
</tr>
</tbody>
</table>

Preparation of Reagent 5 working solution: dilute the Reagent 5 with double distilled water at the ratio of 1: 9 and mix fully. Prepare the fresh solution before use.

Experimental instrument
Test tube, Micropipettor, 37°C water bath, Vortex mixer, Microplate reader (505 nm)
Sample preparation

1. Serum (plasma) and other liquid sample: Detect the sample directly.
2. Animal tissue sample: Accurately weigh the tissue sample, add 9 times the volume of PBS (0.01 M, pH7~7.4) 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, E-BC-K168, E-BC-K165).
3. Culture cell sample: Wash the cells with PBS (0.01 M, pH7~7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add PBS at a ratio of cell number (10^6): PBS (μL) =1: 300-500. Sonicate or grind with hand-operated 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, E-BC-K168, E-BC-K165).

Operation steps

1. The preparation of standard curve

Set 6 wells of micro-plate for standard and operate according to the following operating table.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1 (mL)</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent 2 (mL)</td>
<td>0</td>
<td>0.05</td>
<td>0.10</td>
<td>0.15</td>
<td>0.20</td>
<td>0.25</td>
</tr>
<tr>
<td>Reagent 3 (mL)</td>
<td>0.50</td>
<td>0.45</td>
<td>0.40</td>
<td>0.35</td>
<td>0.30</td>
<td>0.25</td>
</tr>
<tr>
<td>Reagent 4 (mL)</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Mix fully and incubate at 37℃ for 20 min.

| Reagent 5 working solution (mL) | 5 | 5 | 5 | 5 | 5 | 5 |

Mix fully and stand for 15 min at room temperature. Set spectrophotometry to zero with double-distilled water and measure the OD value of each tube with 1 cm optical path cuvette at 505 nm.

2. The measurement of samples

<table>
<thead>
<tr>
<th></th>
<th>Control well</th>
<th>Sample well</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 3 (mL) (pre-heated at 37℃ for 10 min)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Sample (mL)</td>
<td></td>
<td>0.1</td>
</tr>
</tbody>
</table>

Mix fully, then incubate at 37℃ for 30 min.

| Reagent 4 (mL) | 0.5 | 0.5 |
| Sample (mL)    | 0.1 |    |

Mix fully and incubate at 37℃ for 20 min.

| Reagent 5 working solution (mL) | 5 | 5 |

Mix fully and stand for 15 min at room temperature. Set spectrophotometry to zero with double-distilled water and measure the OD value of each tube with 1 cm optical path cuvette at 505 nm.

Note: Steps 1 and 2 can be progress at the same time.
Calculation of results

1. **Definition of international unit:** The enzyme amount of 1 μmol of NADH consumed in reaction system (1 mL sample or 1 g tissue protein, 25°C) per minute is defined as 1 unit (wavelength is 340 nm, optical path is 1 cm).

2. **Definition of carman unit:** 1 mL of sample, the total volume of reaction is 3 mL, wavelength is 340 nm, optical path is 1 cm, react at 25°C for 1 min, the amount of generated pyruvic acid which oxidize NADH to NAD⁺ and cause absorbance decreasing 0.001 is as 1 unit. (1 **carman unit** = 0.482 IU/L, 25°C).

3. Plot the standard curve by using OD value of standard and correspondent carman unit (0, 28, 57, 97, 150, 200 carman unit) as x-axis and y-axis respectively. Create the standard curve with graph software (or EXCEL). The carman unit of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is $y=ax^2+bx+c$.

   1) **Serum/plasma:**
   \[
   \text{ALT/GPT activity (IU/L)} = \left[a \times (\text{OD}_{\text{sample}} - \text{OD}_{\text{control}}) \right]^2 + b(\text{OD}_{\text{sample}} - \text{OD}_{\text{control}}) + c \times 0.482 \text{ IU/L} \times f
   \]

   2) **Tissue and Cells:**
   \[
   \text{ALT/GPT activity (IU/gprot)} = \left[a \times (\text{OD}_{\text{sample}} - \text{OD}_{\text{control}}) \right]^2 + b(\text{OD}_{\text{sample}} - \text{OD}_{\text{control}}) + c \times 0.482 \text{ IU/L} \times f \div Cpr
   \]

   y: carman unit.
   x: the absolute OD value of standard at 505 nm.
   a, b, c: the constant of standard curve.
   f: dilution factor of sample before tested.
   Cpr: concentration of protein in sample (gprot/L)

Technical parameter

1. The sensitivity of the kit is 1.26 IU/L.
2. The intra-assay CV is 4.3% and the inter-assay CV is 8.2%.
3. The detection range of the kit is 1.26-72.3 IU/L.

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. ALT/GPT for serum samples can be stored at room temperature(25 °C) for 2 days, at 4°C for 1 week, -20°C for 1 month.
Appendix: Standard curve

(This is for reference only.)

Result

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average OD value of standard</td>
<td>0.2735</td>
<td>0.3865</td>
<td>0.4970</td>
<td>0.5850</td>
<td>0.6740</td>
<td>0.7495</td>
</tr>
<tr>
<td>Absolute OD</td>
<td>0</td>
<td>0.1130</td>
<td>0.2235</td>
<td>0.3115</td>
<td>0.4005</td>
<td>0.4760</td>
</tr>
<tr>
<td>Corresponding carman unit</td>
<td>0</td>
<td>28</td>
<td>57</td>
<td>97</td>
<td>150</td>
<td>200</td>
</tr>
</tbody>
</table>

Standard curve (Reference)

\[
y = 629.84 x^2 + 115.13 x + 2.0232 \\
R^2 = 0.99779
\]