Overview

Product name: Alanine Transaminase Activity Assay Kit (Colorimetric/Fluorometric) ab105134

Detection method: Colorimetric/Fluorometric

Sample type: Cell culture supernatant, Urine, Serum, Plasma, Other biological fluids, Tissue Extracts, Cell culture media

Assay type: Enzyme activity (quantitative)

Sensitivity: > 10 mU/well

Assay time: 1h 20m

Product overview:
Alanine Transaminase Activity Assay Kit (Colorimetric/Fluorometric) ab105134 is a rapid and simple assay used to quantify alanine transaminase (ALT) activity in mammalian samples.

In the ALT assay protocol, ALT transfers an amino group from alanine to α-ketoglutarate; producing pyruvate and glutamate. The pyruvate is detected in a reaction that converts a nearly colorless probe to a form that is colored (ODmax = 570 nm) and fluorescent (Ex/Em = 535/587 nm).

The kit has a detection limit of 10 mU per well.

ALT assay protocol summary:
- add samples and standards to wells
- add reaction mix and incubate for 10 min at 37°C
- analyze every 2-3 min for 60 min with microplate reader in kinetic mode at 37°C

Notes:
Alanine transaminase is also called alanine aminotransferase or serum glutamic pyruvic transaminase (ALT, ALAT, SGPT).

Platform: Microplate reader

Properties

Storage instructions: Store at -20°C. Please refer to protocols.
Serum aspartate transaminase (AST) and alanine transaminase (ALT) levels were measured at various time periods post Con A injection. Mean values ± SD are shown (n = 4). □P < 0.05 and ■■P < 0.01.

Liver samples from high fat diet (HFD) and standard carbohydrate diet (CHD) BALB/c and C57BL6/J mice were homogenised in an ALT assay buffer for the determination of ALT activity using ab105134. A separate batch of liver extracts was prepared and incubated in a buffer containing NP40 (5%) and supernatants containing the triglycerides were separated. Triglycerides concentration was determined on the supernatant fraction using ab65336. ALT activity and triglycerides concentration were determined by measuring OD at 570nm.

### Components

<table>
<thead>
<tr>
<th>Components</th>
<th>Identifier</th>
<th>100 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT Positive Control (lyophilized)</td>
<td>Blue</td>
<td>1 vial</td>
</tr>
<tr>
<td>ALT Substrate (lyophilized)</td>
<td>Orange</td>
<td>1 vial</td>
</tr>
<tr>
<td>Pyruvate Standard (100 nmol/µl)</td>
<td>Yellow</td>
<td>1 x 100µl</td>
</tr>
<tr>
<td>ALT Assay Buffer</td>
<td>WM</td>
<td>1 x 25ml</td>
</tr>
<tr>
<td>ALT Enzyme Mix (lyophilized)</td>
<td>Green</td>
<td>1 vial</td>
</tr>
<tr>
<td>OxiRed™ (in DMSO)</td>
<td>Red</td>
<td>1 x 200µl</td>
</tr>
</tbody>
</table>
Colorimetric standard curve: mean of duplicates (+/- SD) with background reads subtracted.

Fluorometric standard curve: mean of duplicates (+/- SD) with background reads subtracted.

Alanine transaminase measured in mouse tissue lysates showing quantity (mU) per mg of tested sample.

Protein concentration for samples varied from 4 mg/mL to 13 mg/mL. Samples were diluted 9-27 fold and measured colorimetrically.
Pyruvate measured colorimetrically in cell lysate after 20 min and 40 min incubation time showing quantity (nmol) per 1 mln of tested cells.

Measurement of alanine transaminase in HepG2 cells (10 μg) and liver lysate (15 μg).

Pyruvate measured fluorometrically in cell lysate after 20 min and 40 min incubation time showing quantity (nmol) per 1 mln of tested cells.
Pyruvate measured in biological fluids after 20 min and 40 min incubation time showing quantity (nmol) per ml of tested sample.

Pyruvate measured in mouse tissue lysates after 20 min and 40 min incubation time showing quantity (nmol) per mg of tested sample.

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