Alanine Transaminase Colorimetric Activity Assay Kit

Item No. 700260

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA
GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. For best results, remove components and store as stated below.

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item</th>
<th>Quantity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>700261</td>
<td>ALT Assay Buffer (10X)</td>
<td>1 vial</td>
<td>-20°C</td>
</tr>
<tr>
<td>700262</td>
<td>ALT Substrate</td>
<td>1 vial</td>
<td>-20°C</td>
</tr>
<tr>
<td>700263</td>
<td>ALT Cofactor</td>
<td>2 vials</td>
<td>-20°C</td>
</tr>
<tr>
<td>700264</td>
<td>ALT Initiator</td>
<td>1 vial</td>
<td>-20°C</td>
</tr>
<tr>
<td>700265</td>
<td>ALT Positive Control</td>
<td>1 vial</td>
<td>-80°C</td>
</tr>
<tr>
<td>400014</td>
<td>96-Well Plate (Colorimetric Assay)</td>
<td>1 plate</td>
<td>RT</td>
</tr>
<tr>
<td>400012</td>
<td>96-Well Cover Sheet</td>
<td>1 cover</td>
<td>RT</td>
</tr>
</tbody>
</table>

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.
Safety Data
This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

Precautions
Please read these instructions carefully before beginning this assay.

If You Have Problems
Technical Service Contact Information
Phone: 888-526-5351 (USA and Canada only) or 734-975-3888
Fax: 734-971-3641
Email: techserv@caymanchem.com
Hours: M-F 8:00 AM to 5:30 PM EST
In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability
This kit will perform as specified if stored as directed in the Materials Supplied section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied
1. A plate reader capable of measuring absorbance at 340 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

Background
Alanine transaminase (ALT), also known as alanine aminotransferase (ALAT) or serum glutamic pyruvic transaminase (sGPT), is a homodimeric cytoplasmic pyridoxal phosphate-dependent enzyme involved in cellular nitrogen metabolism, amino acid metabolism, and liver gluconeogenesis. ALT mediates conversion of major intermediate metabolites, catalyzing reversible transamination between alanine and α-ketoglutarate to form pyruvate and glutamate.

ALT is widely distributed in many tissues but is found in greatest abundance in the liver, and to a much lesser extent in the kidneys, heart, and brain. The major role of ALT in the liver is the conversion of alanine to glucose which is then exported to the body to be utilized in a multitude of processes. ALT has also been found to play an important role in neuronal function by supplying an important source of neuronal glutamate through the alanine-aminotransferase reaction.

Serum ALT levels are generally low, but may spike during disease states or in the event of tissue injury. As such, ALT levels are routinely used as indicators of medical issues, particularly liver diseases. Increased levels can be seen in patients with diabetes, cirrhosis, fatty liver disease, and hepatitis. Beyond liver disease, increased ALT levels have been noted in cases of carcinoma, mononucleosis, muscular dystrophy, and cardiovascular disease.
About This Assay

Cayman’s Alanine Transaminase Colorimetric Activity Assay Kit provides a convenient method of detecting ALT activity in serum, plasma, tissue samples, and cell lysates. Measurement of the ALT activity is carried out by monitoring the rate of NADH oxidation in a coupled reaction system employing lactate dehydrogenase (LDH) (see Figure 1). The oxidation of NADH to NAD$^+$ is accompanied by a decrease in absorbance at 340 nm. Under circumstances in which the ALT activity is rate limiting, the rate decrease is directly proportional to the ALT activity in the sample.

Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. ALT Assay Buffer (10X) - (Item No. 700261)
   Dilute 4 ml of Assay Buffer concentrate with 36 ml of HPLC-grade water. This final Buffer (100 mM Tris-HCl, pH 7.8, 10 mM Sodium Bicarbonate, 0.1 mM pyridoxal-5-phosphate, 0.01% sodium azide) should be used in the assay and for reconstituting the substrate and cofactor. This diluted buffer is stable for six months when stored at 4°C.

2. ALT Substrate - (Item No. 700262)
   The vial contains crystalline L-alanine. Dissolve the entire contents of the vial in 30 ml of the diluted Assay Buffer. This is sufficient substrate to assay an entire plate. This solution is stable for one month when stored at 4°C.

3. ALT Cofactor - (Item No. 700263)
   The vial contains a lyophilized powder of NADH and LDH. Immediately prior to assaying, dissolve the entire contents of one vial with 1.5 ml of diluted Assay Buffer. This is enough cofactor to assay 75 wells. Reconstitute two vials if the entire plate is to be run. The reconstituted Cofactor is stable for four hours when stored on ice.

4. ALT Initiator - (Item No. 700264)
   The vial contains 3 ml of 150 mM $\alpha$-ketoglutarate. The reagent is ready to use as supplied. This reagent is stable for six months when frozen at -20°C and five days when stored at 4°C.

5. ALT Positive Control - (Item No. 700265)
   This vial contains lyophilized porcine heart ALT. Reconstitute the contents of the vial with 2 ml of diluted Assay Buffer. The diluted enzyme is stable for one month when frozen at -80°C and five days when stored at 4°C. Avoid repeated freeze/thaw cycles.
Sample Preparation

Plasma

Typically, normal human plasma has ALT concentrations in the range of 8-40 U/L.\(^5\)

1. Collect blood using an anticoagulant such as heparin or citrate.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4\(^{\circ}\)C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice. If not assaying the same day, freeze at -80\(^{\circ}\)C. The plasma sample will be stable for one month while stored at -80\(^{\circ}\)C. Repeated freeze/thaw cycles should be avoided.
3. Plasma does not need to be diluted before assaying.

Serum

Typically, normal human serum has ALT concentrations in the range of 8-40 U/L.\(^5\)

1. Collect blood without using an anticoagulant such as heparin or citrate.
2. Allow blood to clot for 30 minutes at 25\(^{\circ}\)C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4\(^{\circ}\)C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, freeze at -80\(^{\circ}\)C. The serum sample will be stable for one month while stored at -80\(^{\circ}\)C. Repeated freeze/thaw cycles should be avoided.
4. Serum does not need to be diluted before assaying.

Tissue Homogenate

1. Prior to dissection, rinse the tissue with a PBS (phosphate buffered saline) solution, pH 7.4, to remove any red blood cells and clots.
2. Homogenize the tissue in 5-10 ml of cold buffer (i.e., 100 mM Tris, pH 7.8) per gram of tissue.
3. Centrifuge at 10,000 x g for 15 minutes at 4\(^{\circ}\)C.
4. Remove the supernatant and store on ice. If not assaying on the same day, store the sample at -20\(^{\circ}\)C. The sample will be stable for at least one month when frozen.

**NOTE:** If the rate of $A_{340}$ decrease is greater than 0.04 Abs units/min, dilution of the sample with diluted assay buffer will be necessary to fall within the linear range of the assay.

Cell Lysate

1. Collect cells (~5 x 10\(^6\)) by centrifugation (i.e., 1,000-2,000 x g for 10 minutes at 4\(^{\circ}\)C). For adherent cells, do not proteolytic enzymes, rather use a rubber policeman.
2. Homogenize the cell pellet in 0.5-1.0 ml cold buffer (i.e., 100 mM Tris, pH 7.5, 1 mM EDTA).
3. Centrifuge at 10,000 x g for 15 minutes at 4\(^{\circ}\)C.
4. Remove the supernatant and store on ice. If not assaying on the same day, freeze the sample at -80\(^{\circ}\)C. The sample should be stable for at least one month.

**NOTE:** If the rate of $A_{340}$ decrease is greater than 0.04 Abs units/min, dilution of the sample with diluted assay buffer will be necessary to fall within the linear range of the assay.
**Plate Set Up**

There is no specific pattern for using the wells on the plate. It is recommended that three wells be designated for the Positive Control. It is suggested that each sample be assayed in triplicate and that you record the contents of each well on the template sheet provided on page 18. A typical layout of samples to be measured in triplicate is given below.

![Sample plate format](image)

+ = ALT Positive Control  
S1-S31 = Sample Wells

Figure 2. Sample plate format

**Pipetting Hints**

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

**General Information**

- The final volume of the assay is 210 µl in all the wells.
- Use the diluted Assay Buffer in the assay.
- All reagents must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- We recommend assaying samples in triplicate, but it is the user’s discretion to do so.
- 31 samples can be assayed in triplicate or 47 in duplicate.
- The assay is performed at 37°C.
- Monitor the absorbance at 340 nm.
Performing the Assay

1. **Positive Control Wells** - add 150 µl of Substrate, 20 µl of Cofactor, and 20 µl of Positive Control.
2. **Sample Wells** - add 150 µl of Substrate, 20 µl of Cofactor, and 20 µl of sample.
3. Place the plate cover over the plate and incubate at 37°C for 15 minutes.
4. Remove the plate cover and initiate the reactions by adding 20 µl of ALT Initiator to all the wells being used as quickly as possible.
5. Immediately begin reading the plate at 340 nm once every minute for a period of five minutes.

**NOTE:** Background Wells (optional) - the background activity is typically insignificant in the evaluation of ALT activity in a sample. However, if desired, a background value can be obtained for each sample. For each sample being assayed, add 150 µl of diluted Assay Buffer without the ALT Substrate, 20 µl of sample, 20 µl of ALT Cofactor, and 20 µl of ALT Initiator. Read the absorbance at 340 nm every minute for a period of five minutes.

Calculations

1. Determine the change in absorbance ($\Delta A_{340}$) per minute by:
   a. Plotting the absorbance values as a function of time to obtain the slope (rate) of the linear portion of the curve (a graph is shown using porcine heart alanine transaminase, see Figure 3, page 14).
   OR
   b. Select two points on the linear portion of the curve and determine the change in absorbance during that time using the following equation:

   $$\frac{\Delta A_{340}/\text{min.}}{\text{Time 2 (min.)} - \text{Time 1 (min.)}} = \frac{A_{340} (\text{Time 2}) - A_{340} (\text{Time 1})}{\text{Time 2 (min.)} - \text{Time 1 (min.)}}$$

   *Use the absolute value.

2. If running background wells, determine the rate of $\Delta A_{340}$ for the background and subtract this rate from that of the sample wells. The reaction rate at 340 nm can be determined using the NADH extinction coefficient of 4.11 mM$^{-1}$. One unit is defined as the amount of enzyme that will cause the oxidation of 1.0 µmol of NADH to NAD$^+$ per minute at 37°C.

3. Use the following formula to calculate the ALT activity.

   $$\text{ALT activity (U/ml)} = \left[ \frac{\Delta A_{340}/\text{min} \times 0.21 \text{ ml}}{4.11 \text{ mM}^{-1} 	imes 0.02 \text{ ml}} \right] \times \text{Sample dilution}$$

   *The actual extinction coefficient for NADH at 340 nm is 6.22 mM$^{-1}$cm$^{-1}$. This value has been adjusted for the pathlength of the solution in the well (0.66 cm).

   **NOTE:** To convert to SI units (IU) or nKat/ml, multiply U/ml by a factor of 16.67. To convert to U/L, multiply U/ml by 1,000.
Figure 3. Activity of porcine heart alanine transaminase

Performance Characteristics

Sensitivity:
The limit of detection for this assay is 0.006 U/ml, or 0.1 SI U/ml.

Precision:
When a series of 77 ALT measurements were performed on the same day under the same experimental conditions, the intra-assay coefficient of variation was 5.8%. When a series of ten samples were performed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 7.8%.

Interferences

The following reagents were tested in the assay for interference in the assay:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Will Interfere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffers</td>
<td></td>
</tr>
<tr>
<td>Tris</td>
<td>No</td>
</tr>
<tr>
<td>HEPES</td>
<td>No</td>
</tr>
<tr>
<td>MES</td>
<td>Yes</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Yes</td>
</tr>
<tr>
<td>Detergents</td>
<td></td>
</tr>
<tr>
<td>Polysorbate 20 (0.1%)</td>
<td>No</td>
</tr>
<tr>
<td>Polysorbate 20 (1%)</td>
<td>No</td>
</tr>
<tr>
<td>Triton X-100 (0.1%)</td>
<td>No</td>
</tr>
<tr>
<td>Triton X-100 (1%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Chelators</td>
<td></td>
</tr>
<tr>
<td>EDTA (1 mM)</td>
<td>No</td>
</tr>
<tr>
<td>EGTA (1 mM)</td>
<td>No</td>
</tr>
<tr>
<td>Protease Inhibitors/Enzymes</td>
<td></td>
</tr>
<tr>
<td>Trypsin (10 μg/ml)</td>
<td>Yes</td>
</tr>
<tr>
<td>PMSF (200 μM)</td>
<td>Yes</td>
</tr>
<tr>
<td>Leupeptin (10 μg/ml)</td>
<td>No</td>
</tr>
<tr>
<td>Antipain (10 μg/ml)</td>
<td>No</td>
</tr>
<tr>
<td>Chymostatin (10 μg/ml)</td>
<td>No</td>
</tr>
<tr>
<td>Solvents</td>
<td></td>
</tr>
<tr>
<td>Ethanol (5%)</td>
<td>Yes</td>
</tr>
<tr>
<td>Methanol (5%)</td>
<td>No</td>
</tr>
<tr>
<td>Dimethylsulfoxide (5%)</td>
<td>No</td>
</tr>
<tr>
<td>Others</td>
<td></td>
</tr>
<tr>
<td>BSA (0.1%)</td>
<td>No</td>
</tr>
<tr>
<td>Glutathione (1 mM)</td>
<td>No</td>
</tr>
<tr>
<td>Glycerol (10%)</td>
<td>No</td>
</tr>
<tr>
<td>Problem</td>
<td>Possible Causes</td>
</tr>
<tr>
<td>---------</td>
<td>----------------</td>
</tr>
</tbody>
</table>
| Erratic values; dispersion of duplicates/triplicates | A. Poor pipetting/technique  
B. Bubble in the well(s) | A. Be careful not to splash the contents of the wells  
B. Carefully tap the side of the plate with your finger to remove bubbles |
| No decrease in absorbance but has a high initial absorbance (~0.5) | A. Sample was not added to the wells  
B. ALT activity is too low to detect | A. Make sure to add all the components to the wells and re-assay  
B. Concentrate the sample with an Amicon concentrator with a MW cut-off of 10 kDa and re-assay |
| No decrease in absorbance but has a low initial absorbance (<0.2) | Little or no cofactor was added to the well in question | Make sure to add all the components to the wells and re-assay |

**References**

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions.

Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

This document is copyrighted. All rights are reserved. This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine-readable form without prior consent, in writing, from Cayman Chemical Company.

©06/27/2017, Cayman Chemical Company, Ann Arbor, MI, All rights reserved. Printed in U.S.A.