Alanine Transaminase Colorimetric Activity Assay Kit

Item No. 700260

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. After opening the kit, remove components and store as stated below.

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

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

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 with the ability to measure absorbance at 340 nm
2. Adjustable pipettes and a multichannel pipette
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

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.[1] ALT mediates conversion of major intermediate metabolites, catalyzing reversible transamination between alanine and α-ketoglutarate to form pyruvate and glutamate.[2]

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.[2] 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 analine-aminotransferase reaction.[3]

Serum ALT levels are generally low, but may spike during disease states or in the event of tissue injury.[4] 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.[5-8]

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.

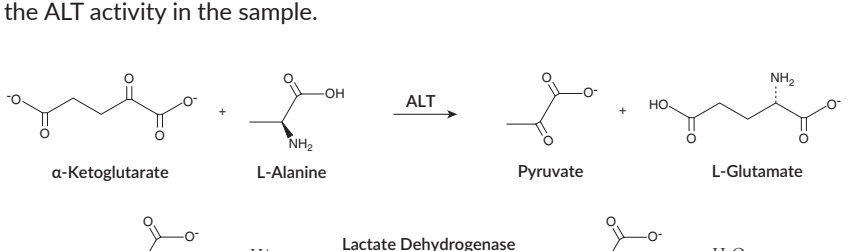


Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **ALT Assay Buffer (10X) - (Item No. 700261)**

   Mix 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)**

   This 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)**

   This vial contains a lyophilized powder of NADH and LDH. Immediately prior to assaying, reconstitute 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 running the full plate. The reconstituted Cofactor is stable for four hours when stored on ice.

4. **ALT Initiator - (Item No. 700264)**

   This vial contains 3 ml of 150 mM α-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°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°C. The plasma sample will be stable for one month while stored at -80°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°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°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°C. The serum sample will be stable for one month while stored at -80°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°C.
4. Remove the supernatant and store on ice. If not assaying on the same day, store the sample at -20°C. The sample will be stable for at least one month when frozen.

NOTE: If the rate of A_{340} decrease is greater than 0.4 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°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°C.
4. Remove the supernatant and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample should be stable for at least one month.

NOTE: If the rate of A_{340} decrease is greater than 0.4 Abs units/min, dilution of the sample with diluted assay buffer will be necessary to fall within the linear range of the assay.

ASSAY PROTOCOL

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.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	PC	PC	S8	S8	S8	S16	S16	S16	S24	S24	S24
B	S1	S1	S1	S9	S9	S9	S17	S17	S17	S25	S25	S25
C	S2	S2	S2	S10	S10	S10	S18	S18	S18	S26	S26	S26
D	S3	S3	S3	S11	S11	S11	S19	S19	S19	S27	S27	S27
E	S4	S4	S4	S12	S12	S12	S20	S20	S20	S28	S28	S28
F	S5	S5	S5	S13	S13	S13	S21	S21	S21	S29	S29	S29
G	S6	S6	S6	S14	S14	S14	S22	S22	S22	S30	S30	S30
H	S7	S7	S7	S15	S15	S15	S23	S23	S23	S31	S31	S31

PC = ALT Positive Control
S1-S31 = Sample Wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette 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 to the designated wells on the plate.
2. **Sample Wells -** add 150 µl of Substrate, 20 µl of Cofactor, and 20 µl of sample to the designated wells on the plate.
3. Cover plate and incubate at 37°C for 15 minutes.
4. Remove the plate cover and quickly initiate the reactions by adding 20 µl of ALT Initiator to all the wells being used.
5. Immediately measure the absorbance at 340 nm once every minute for 10 minutes at 37°C. If ALT activity is low, continue reading for up to 60 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 to the designated wells on the plate. Immediately measure the absorbance at 340 nm once every minute for 10 minutes at 37°C. If ALT activity is low, continue reading for up to 60 minutes.*

ANALYSIS

Calculations

1. Determine the change in absorbance (ΔA_{340}) per minute by:
   a. Plot the absorbance values as a function of time to obtain the slope (rate) of the linear protion 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:

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

*Use the absolute value.

2. If running background wells, determine the rate of ΔA_{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.

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

*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.

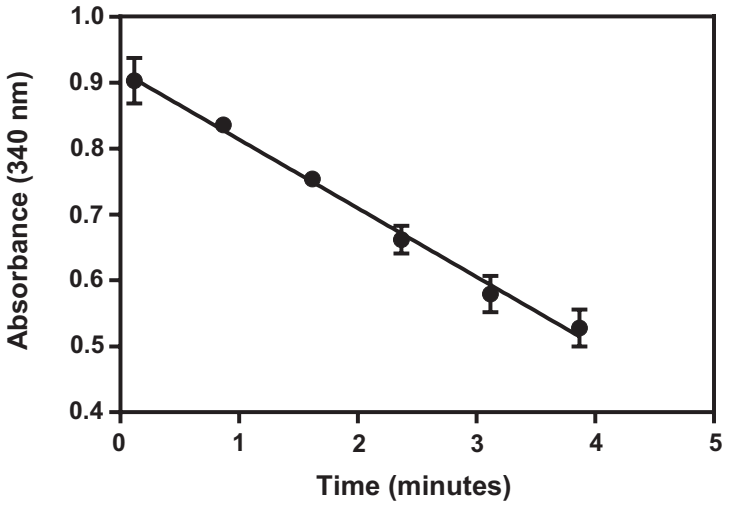


Figure 3. Activity of porcine heart alanine transaminase

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%.

RESOURCES

Interferences

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

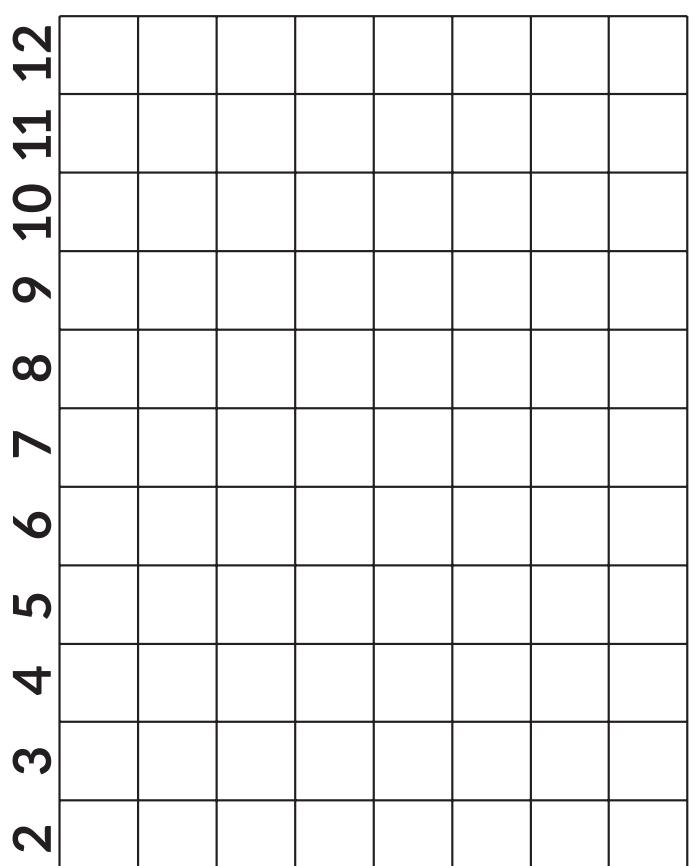
Reagent		Will Interfere (Yes or No)
Buffers	Tris	No
	HEPES	No
	MES	Yes
	Phosphate	Yes
Detergents	Polysorbate 20 (0.1%)	No
	Polysorbate 20 (1%)	No
	Triton X-100 (0.1%)	No
	Triton X-100 (1%)	Yes
Chelators	EDTA (1 mM)	No
	EGTA (1 mM)	No
Protease Inhibitors/ Enzymes	Trypsin (10 µg/ml)	Yes
	PMSF (200 µM)	Yes
	Leupeptin (10 µg/ml)	No
	Antipain (10 µg/ml)	No
	Chymostatin (10 µg/ml)	No
Solvents	Ethanol (5%)	Yes
	Methanol (5%)	No
	Dimethylsulfoxide (5%)	No
Others	BSA (0.1%)	No
	Glutathione (1 mM)	No
	Glycerol (10%)	No

Troubleshooting

Problem	Possible Causes	Recommended Solutions
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

1. Ishiguro, M., Takio, K., Suzuki, M., *et al.* Complete amino acid sequence of human liver cytosolic alanine aminotransferase (GPT) determined by a combination of conventional and mass spectral methods. *Biochemistry* **30**, 10451-10457 (1991).
2. Yang, R.-Z., Park, S., Reagan, W.J., *et al.* Alanine aminotransferase isoenzymes: Molecular cloning and quantitative analysis of tissue expression in rats and serum elevation in liver toxicity. *Hepatology* **49**, 598-607 (2009).
3. Erecinska, M., Nelson, D., Nissim, I., *et al.* Cerebral alanine transport and alanine aminotransferase reaction: Alanine as a source of neuronal glutamate. *J. Neurochem.* **62**, 1953-1964 (1994).
4. Pratt, D.S. and Kaplan, M.M. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N. Engl. J. Med.* **342(17)**, 1266-1271 (2000).
5. Kim, H.C., Nam, C.M., Jee, S.H., *et al.* Normal serum aminotransferase concentration and risk of mortality from liver diseases: Prospective cohort study. *BMJ* (2004).
6. Vozarova, B., Stefan, N., Lindsay, R.S., *et al.* High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* **51**, 1889-1895 (2002).
7. Prati, D., Taioli, E., Zanella, A., *et al.* Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med.* **137**, 1-9 (2002).
8. Tarao, K., Rino, Y., Ohkawa, S., *et al.* Association between high serum alanine aminotransferase levels and more rapid development and higher rate of incidence of hepatocellular carcinoma in patients with hepatitis C virus-associated cirrhosis. *Cancer* **86**, 589-595 (1999).



NOTES

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.