PO Box 1145, Station CSC, London ON N6A 5K2 Canada
Tel: +1 519 434 5057 Fax: +1 519 434 2639
www.idlabs.com idinfo@idlabs.com

IDTox™ Alanine Transaminase (ALT) Color Endpoint Assay Kit

SUP6001-C

Color Endpoint Assay for the determination of the Alanine transaminase enzyme in serum samples.

PRODUCT DESCRIPTION

The ID Labs Alanine Transaminase (ALT) Color Endpoint Assay Kit a plate-based colorimetric enzymatic assay for the determination of the alanine transaminase enzyme in serum samples. Alanine transaminase (ALT) also known as alanine aminotransferase or (sGPT) is a metabolic enzyme expressed primarily in the liver. Elevation of ALT levels is an indication of liver damage and has been associated with liver injury. ALT levels are monitored routinely in patients with liver diseases. ALT is also a very useful tool for preclinical investigation of experimental drug formulations and ALT levels are commonly used to monitor and attenuate the hepatotoxic effects of experimental drugs in rodents.

The kit uses a colorimetric assay to detect changes in alanine transaminase levels directly from serum samples. The features of the kit are:

- High sensitivity and low detection limit (10 U/L)
- Convenient, colorimetric, end-point assay
- Does not require expensive instrumentation
- High reproducibility
- Only 10 µl of serum sample is needed

PROCEDURE OVERVIEW

The Alanine Transaminase (ALT) Color Endpoint Assay Kit uses a colored endpoint reaction to specifically detect ALT enzymatic activity. In this method, alanine and α-ketoglutarate are first converted by the ALT enzyme to glutamate and pyruvate. The pyruvate then reacts with a hydrazine reagent to form a colored product. The concentration of ALT in each sample is determined by measuring the absorbance at 510 nm. Dilutions of the Pyruvate Control, included in the kit, can be used to construct a standard curve to calibrate the assay and confirm assay linearity. For In vitro research use only.

KIT REAGENTS SUPPLIED

The Alanine Transaminase (ALT) Enzymatic Assay Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). The kit also contains enough material to construct four standard curves. Store the kit at 4°C. The shelf life of the kit, when properly stored is noted on the label. For more details, see "Preparation of Reagent Mix"

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	2-8ºC
ALT Reagent Mix	7 ml	2-8ºC
Pyruvate Control	1 tube	2-8ºC
Pyruvate Dilution Buffer	2 x 1.8 ml	2-8ºC
DPNH Color Solution	7 ml	2-8ºC

MATERIALS REQUIRED BUT NOT PROVIDED

Microtiter plate reader (510 nm).
Microfuge to prepare serum samples.
Deionized or distilled water.
0.5M NaOH
1.5 ml microfuge tubes.
Multichannel pipette or repeating pipettor (*recommended but not required*).

For *in vitro* research use. CAUTION: Not for human or animal therapeutic use.
Uses other than the labeled intended use may be a violation of local law.

SENSITIVITY (Serum Detection Limit)
10 U/L

WARNINGS AND PRECAUTIONS
It is strongly recommended that you read the following warnings and precautions to ensure your full awareness of the techniques and other details you should pay close attention to when running the assays. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the protocol coming with the kit.

Do not use the kit past the expiration date.
Try to maintain a laboratory temperature of (20–25°C/68–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should also be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
Make sure to use only distilled or deionized water since water quality is very important.
When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.

ID Labs™ makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. ID Labs™ shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product. This product is For In Vitro Research Use Only.

ASSAY PROCEDURE

PREPARATION OF SAMPLE
Serum

1. Carefully collect whole blood in a 1.5 ml microfuge tube or serum collection tube making sure to avoid hemolysis as it will release erythrocyte ALT enzyme into the serum.
2. Incubate the blood sample at 37°C for 10 minutes.
3. Centrifuge sample at 10,000 rpm for 10 minutes.
4. Transfer serum layer to a clean 1.5ml microfuge tube, avoiding the “buffy coat” layer.
5. Store serum samples on ice or at 4°C prior to testing; do not freeze samples. Serum samples can be stored at 4°C for up to one week.

ASSAY PROCEDURE
Set up

Allow all reagents and the microtitre plate to warm up to room temperature before use (for at least 30 minutes).
Turn on the plate reader, allow light source to warm up, and set the absorbance wavelength to 510 nm.

Reagent Preparation

Preparation of Pyruvate Control Dilutions for Standard Curve

Label six 1.5ml microfuge tubes: 1, 2, 3, 4, 5, Neg. Then make 6 serial dilutions of the Pyruvate Control (3 concentration increments per log) using the Pyruvate Dilution Buffer as described in the table below.

NOTE: There is enough material to construct 4 Standard Curves. Make the Pyruvate Control Dilutions for the Standard Curve fresh each time that the Standard Curve is performed. After each dilution, briefly mix the tube before performing the next dilution.

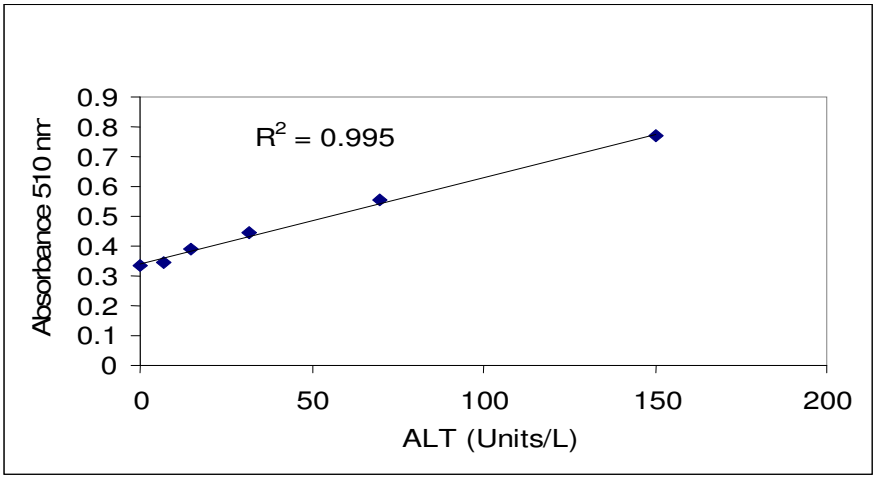
Standard Tube #	Preparation	Equivalent ALT Conc
1	Add 150 µl of Pyruvate Control.	150
2	Add 100 µl from Standard Tube # 1 + 115 µl of Pyruvate Dilution Buffer. Mix thoroughly.	70
3	Add 100 µl from Standard Tube # 2 + 115 µl of Pyruvate Dilution Buffer. Mix thoroughly.	32
4	Add 100 µl from Standard Tube # 3 + 115 µl of Pyruvate Dilution Buffer. Mix thoroughly.	15
5	Add 100 µl from Standard Tube # 4 + 115 µl of Pyruvate Dilution Buffer. Mix thoroughly.	7
6 (Neg)	Add 100 µl of Pyruvate Dilution Buffer.	NA

Sample Test Procedure

1 Add 10 µl of each sample or standard (in duplicate) to the bottom of the microplate wells.
2 Add 50 µl of ALT Reagent Mix to the wells. (Using a multichannel pipet or repeating pipettor is recommended). To ensure mixing, add the reagent directly to the spot in the well where the sample was added.
3 Incubate the plate at 37°C for 30 minutes.
4 Shake DPNH Color Solution before use. Add 50 µl of DPNH Color Solution to each well.
5 Incubate the plate at 37°C for 10 minutes.
6 Add 200 µl 0.5 M NaOH to each well. Incubate the plate at 37°C for 5 minutes
7 Measure the absorbance of each sample at 510 nm.

CALCULATION OF RESULTS

Standard Curve Construction
A standard curve can be constructed using the serially-diluted standards by plotting the average absorbance for each pyruvate standard against its concentration in IU/L.



Calculate the slope and the y-intercept for the line which best fits the standard curve data plot.
The ALT concentration in each sample can be described by the equation:

ALT concentration = (mean absorbance – y-intercept)/slope
Use the mean absorbance values for each serum sample to determine the corresponding concentration of ALT from the standard curve.

References:

Pratap A., et al. "Cyclopamine attenuates acute warm ischemia reperfusion injury in cholestatic rat liver; hope for marginal livers". Mol. Pharmaceutics. 2011.

Pratap A., et al. "Inhibition of endogenous hedgehog signaling protects against acute liver injury after ischemia reperfusion". Pharm Res. 27:2492-2504. 2010.