BIOO SCIENTIFIC
MAXIMIZE SCIENCE FOR LIFE®

BIOO RESEARCH PRODUCTS

MAX DISCOVERY™

Alanine Transaminase (ALT) Enzymatic Assay Kit Manual

Catalog #: 3460-01

TABLE OF CONTENTS

GENERAL INFORMATION 1

Product Description 1
Procedure Overview 1
Kit Contents, Storage and Shelf Life 1
Required Materials Not Provided With the Kit 2
Sensitivity (Detection Limit) 2
Warnings and Precautions 2

SAMPLE PREPARATION 2

Serum 2

ALANINE TRANSAMINASE (ALT) DETECTION PROTOCOL 3

Reagent Preparation 3
Assay Protocol 3

DATA ANALYSIS 4

Determination of Alanine Transaminase Activity in Serum Samples 4
Standard Curve Construction (Optional) 4

SELECTED REFERENCES 5

GENERAL INFORMATION

Product Description

The *MaxDiscovery™ Alanine Transaminase (ALT) Enzymatic Assay Kit* is 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. Damage to the liver causes the release of this enzyme into the blood. 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 spectrophotometric, kinetic assay to detect changes in alanine transaminase levels directly from serum samples. The unique features of the kit are:

- High sensitivity and low detection limit (20 U/L)
- A rapid (5 minutes), robust enzyme-based assay which does not require expensive instrumentation
- High reproducibility
- Only requires 10 μL of serum

Procedure Overview

The *MaxDiscovery™ Alanine Transaminase (ALT) Enzymatic Assay Kit* uses a coupled enzymatic reaction scheme: alanine and α-ketoglutarate are first converted to glutamate and pyruvate which is converted by lactate dehydrogenase to make lactate and NAD^+. The conversion of the NADH chromophore to NAD^+ product, measured at 340 nm, is proportional to the level of ALT enzyme in the sample. The absorbance of each well at 340 nm is measured using a plate reader. The concentration of ALT in each sample is then directly determined from the change in absorbance at 340 nm within 5 minutes time. 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. This is described in more detail in Section, “Data Analysis.”

Kit Contents, Storage and Shelf Life

The *MaxDiscovery™ 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 is 12 months when properly stored. Once the Reagent Mix is reconstituted the shelf life of the kit is 3 months when properly stored. For more details, see “Preparation of Reagent Mix”.

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	4ºC
Reagent Mix	bottle	4ºC
Pyruvate Control	1 tube	4ºC
Pyruvate Dilution Buffer	2 x 1.8 mL	4ºC

Required Materials Not Provided With the Kit

- Microtiter plate reader (*340 nm*)
- Centrifuge (*to prepare serum samples*)
- Deionized or distilled water
- 1.5 mL microfuge tubes
- Multichannel pipet or repeating pipettor (*Optional*)

Sensitivity (Detection Limit)

Sample Type	**Detection Limit** (U/L)
Serum	20

Warnings and Precautions

BIOO strongly recommends 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 included with the kit. If you need further assistance, you may contact your local distributor or BIOO at techsupport2@biooscientific.com.

- 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 be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
- Make sure you are using only distilled 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.

BIOO 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. BIOO shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

SAMPLE PREPARATION

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. Remove serum layer to a clean 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.
6. Use 10 µL of serum in the assay.

ALANINE TRANSAMINASE (ALT) DETECTION PROTOCOL

Reagent Preparation

IMPORTANT: Make sure you read "Warnings and Precautions" section on page 2. ALL REAGENTS AND THE MICROTITER PLATE SHOULD BE BROUGHT UP TO ROOM TEMPERATURE BEFORE USE (30 MIN - 1 HOUR AT 20–25°C/68–77°F).

Preparation of Reagent Mix

To reconstitute the Reagent Mix, add exactly 27 mL of deionized or distilled water to the Reagent Mix powder. Mix by swirling or inverting the bottle 10 times. Allow contents to dissolve for 10 minutes at room temperature.

IMPORTANT: The reconstituted Reagent Mix can be left at room temperature for short periods (30 – 60 min) prior to use. Between uses, the reconstituted Reagent Mix should be stored at 4 °C (for up to 3 months). Discard the Reagent Mix 3 months after reconstitution.

To obtain higher sensitivity measurements use a temperature controlled plate reader, if available. Adjust the plate reader temperature control to 37°C and equilibrate the Reagent Mix to 37°C for 10 minutes before use.

Preparation of Pyruvate Control Dilutions for Standard Curve (*Optional*)

Label six 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	Relative Dilution*
1	Add 150 µL of Pyruvate Control.	1
2	Add 100 µL from Standard Tube #1 + 115 µL of Pyruvate Dilution Buffer. Mix thoroughly.	2.15
3	Add 100 µL from Standard Tube #2 + 115 µL of Pyruvate Dilution Buffer. Mix thoroughly.	4.63
4	Add 100 µL from Standard Tube #3 + 115 µL of Pyruvate Dilution Buffer. Mix thoroughly.	10
5	Add 100 µL from Standard Tube #4 + 115 µL of Pyruvate Dilution Buffer. Mix thoroughly.	21.5
6 (Neg)	Add 100 µL of Pyruvate Dilution Buffer.	NA

**Only needed for the generation of the Standard Curve.*

Assay Protocol

1. Add 10 µL of each sample or standard (in duplicate) to the microplate wells.
2. Add 240 µL of Reagent Mix to the wells. (Using a multichannel pipet or repeating pipettor is recommended).
3. Immediately measure the absorbance of each sample at 340 nm. Exactly 5 min later, measure absorbance again.

DATA ANALYSIS

Determination of Alanine Transaminase Activity in Serum Samples

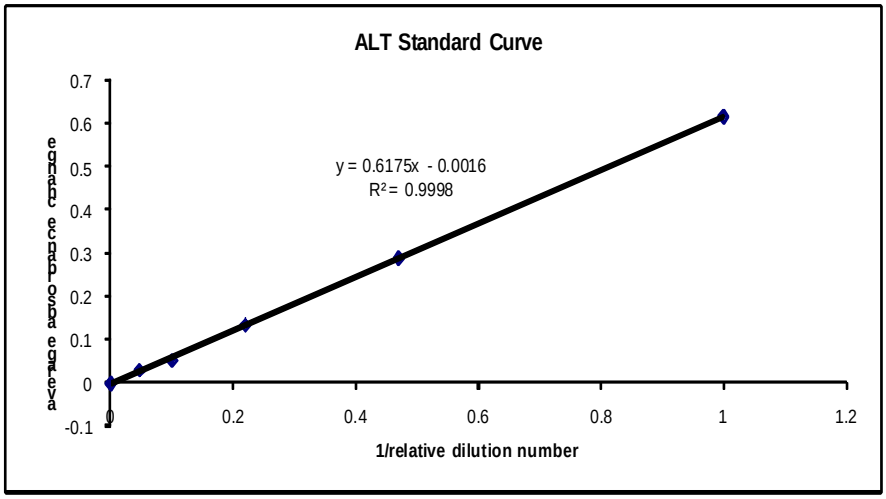
Using the supplied materials and the procedure described above (for measurements performed at 37°C), the concentration of ALT (units per liter) can be determined by multiplying the decrease in absorbance in 5 min by 1072.

For example, if an absorbance decrease of 0.1 is observed over the 5 min interval, the ALT enzyme concentration in the sample would be 1072 x 0.1 = 107.2 U/L.

Standard Curve Construction *(Optional)*

A calibration curve to confirm assay linearity can be constructed using the Pyruvate Control Dilutions as described below:

1. For each calibration point, calculate the *average absorbance change.* To do this, subtract the average **5 min** absorbance value of each point from the average **5 min** absorbance value of the "**Neg**" (no pyruvate) point. This calculation should include subtracting the average 5 min absorbance of the "Neg" value from itself, which is approximately zero.
2. For each standard, plot the average absorbance change along the y-axis (from lowest in value to highest in value) and the inverse value of the relative dilution number* (i.e. 0.047, 0.1, 0.22, 0.47 and 1) on the x-axis. For Tube #6 (Neg) use "0".



*Relative dilution numbers can be found in the table on page 3.

Selected References

Fang X, Wang R, Ma J, Ding Y, Shang W, et al. (2012) Ameliorated ConA-Induced Hepatitis in the Absence of PKC-theta. PLoS ONE 7(2): e31174. doi:10.1371/journal.pone.0031174

Hu, S. et al. (July 6, 2011) ^{13}C-Pyruvate Imaging Reveals Alterations in Glycolysis that Precede c-*Myc*-Induced Tumor Formation and Regression. Cell Metabolism 14 (1): 131 – 142.

Kühnel, F. et al. (December 29, 2009) Targeting of p53-Transcriptional Dysfunction by Conditionally Replicating Adenovirus Is Not Limited by p53-Homologues. Mol. Ther. http://www.nature.com/mt/journal/vaop/ncurrent/full/mt2009298a.html

Kverka, M. et al. (May 2011) Safety and efficacy of the immunosuppressive agent 6-tioguanine in murine model of acute and chronic colitis, *BMC Gastroenterology*, 11 (1) 47.

Venkatesh, M., et al. (July, 2011) In Vivo and In Vitro Characterization of a First-in-Class Novel Azole Analog That Targets Pregnane X Receptor Activation. Molecular Pharmacology 80(1): 124-135.

Wu, S.Y. et al. (August 12, 2010) Systemic delivery of E6/7 siRNA using novel lipidic particles and its application with cisplatin in cervical cancer mouse models. Gene Therapy. http://www.nature.com/gt/journal/vaop/ncurrent/full/gt2010113a.html

Xia, M. et al. (2011) Immune Activation Resulting From NKG2D/Ligand Interaction Promotes Atherosclerosis. Circulation. doi: 10.1161/CIRCULATIONAHA.111.034850

BIOO SCIENTIFIC
MAXIMIZE SCIENCE FOR LIFE®

Bioo Scientific Corporation
3913 Todd Lane Suite 312
Austin, TX 78744 USA
Tel: 1.888.208.2246
Fax: (512) 707-8122

Made in USA
BIOO Research Products Group
info@biooscientific.com
techsupport2@biooscientific.com
www.biooscientific.com