Alanine Transaminase Activity Microplate Assay Kit

User Manual

Catalog # CAK1002

(Version 1.4F)

Detection and Quantification of Alanine Transaminase (ALT) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Alanine Transaminase (ALT), also known as serum alanine aminotransferase (ALAT) or pyruvic transaminase (GPT), facilitates the conversion of alanine and α-ketoglutarate to pyruvate and glutamate. ALT plays an important role in gluconeogenesis and amino acid metabolism. ALT is found mainly in the liver, and, to a lesser extent, in kidney, heart, muscle, and pancreas tissues. Normal serum levels of ALT are low, and increased serum ALT activity is widely used as a marker for liver damage.

Alanine Transaminase Activity Microplate Assay Kit provides a simple and direct procedure for measuring alanine transaminase activity in a variety of samples. This kit is based on the hydrolysis of substrate, reaction product phenylhydrazone can be measured at a colorimetric readout at 520 nm, which is directly proportional to the amount of alanine transaminase activity.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	4 °C
Dye Reagent I	5 ml x 1	4 °C
Dye Reagent II	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 10 ml Assay Buffer to dissolve before use. Store at -20 °C. Use within one month.

Standard: centrifuge the tube briefly, add 1 ml Assay Buffer to dissolve before use, it will be 20 µmol/ml. Store at -20 °C. Use within one month.

Positive Control: centrifuge the tube briefly, add 0.1 ml assay buffer to dissolve before use. Store at -80 °C. Use within one month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 520 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Sample	10 µl	--	--	--	--
Assay Buffer	--	10 µl	--	--	--
Standard	--	--	10 µl	--	--
Distilled Water	--	--	--	10 µl	--
Positive Control	--	--	--	--	10 µl
Substrate	50 µl	50 µl	50 µl	50 µl	50 µl
Mix, cover the plate adhesive strips, put it into the oven, 37 °C for 30 minutes.					
Dye Reagent I	50 µl	50 µl	50 µl	50 µl	50 µl
Dye Reagent II	90 µl	90 µl	90 µl	90 µl	90 µl
Mix, record absorbance measured at 520 nm.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One Unit of ALT activity is defined as the enzyme produces 1 μmol of pyruvic acid per minute.

1. According to the volume of serum or plasma

$$ALT\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.667 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

$$ALT\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 0.667 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$ALT\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 0.667 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the concentration of standard, 20 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

$V_{Standard}$: the volume of standard, 0.01 ml;

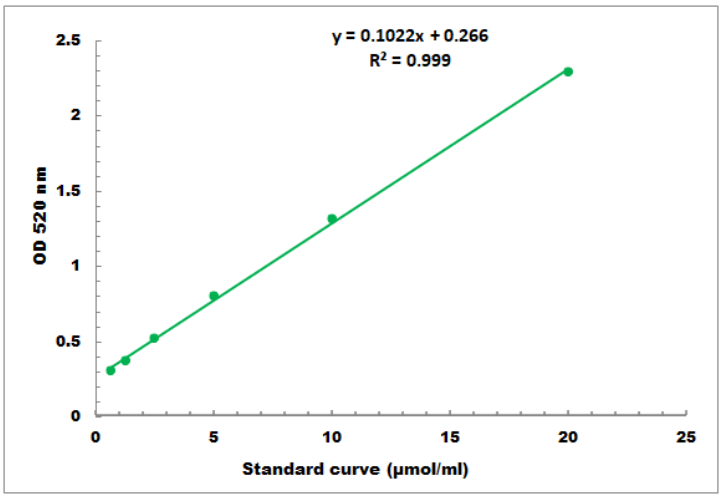
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

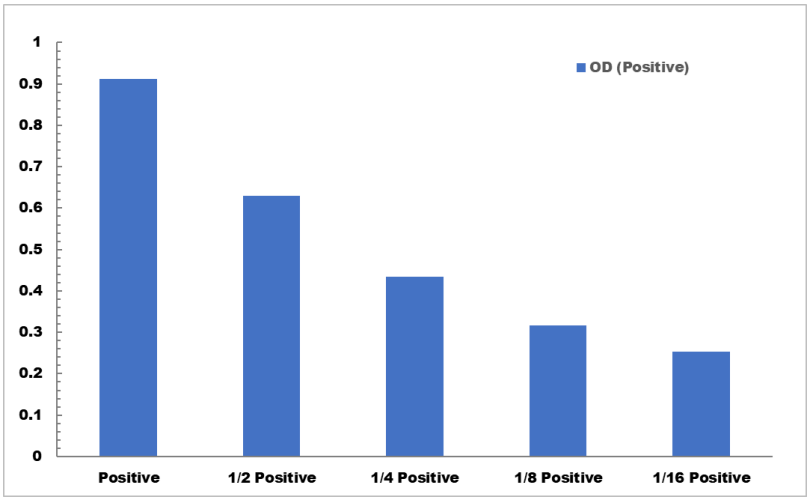
T: the reaction time, 30 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.625 μmol/ml - 20 μmol/ml



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES