



Alanine Transaminase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1002

(Version 1.5G)

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, keep in dark
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	

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. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve to generate 20 $\mu\text{mol/ml}$ as the top standard solution. Store at 4 °C for 1 month after reconstitution. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 20/10/5/2.5/1.25/0.625 $\mu\text{mol/ml}$.

Substrate: Add 5 ml Assay Buffer to dissolve before use. Keep in dark and store at 4°C for 1 week.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use. Store at -20°C.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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.

VI. 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	50µl	50µl	50µl	50µl	50µl
Mix, cover the plate adhesive strips, put it into the oven, 37 °C for 30 minutes.					
Dye Reagent I	50µl	50µl	50µl	50µl	50µl
Dye Reagent II	90 µl	90 µl	90 µl	90 µl	90 µl
Mix, record absorbance measured at 520 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. 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 slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein}: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10⁴

C_{Standard}: the concentration of standard, μmol/mL

V_{Standard}: the volume of standard in assay procedure, mL

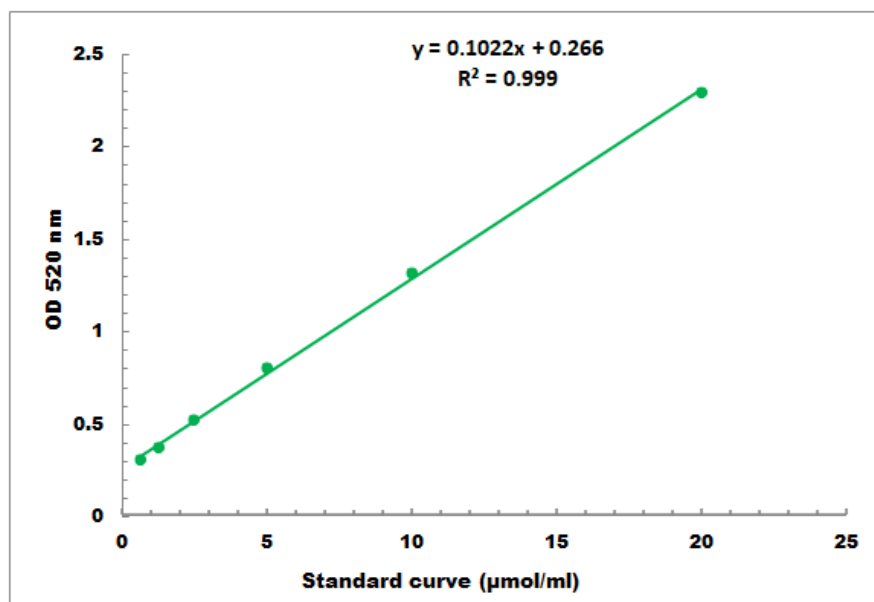
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

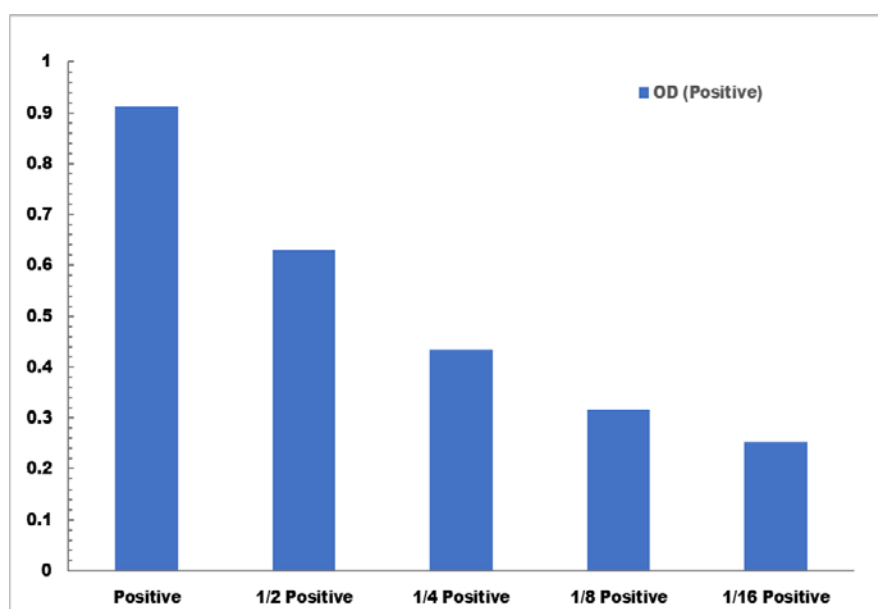
T: the reaction time, minute

VII. TYPICAL DATA

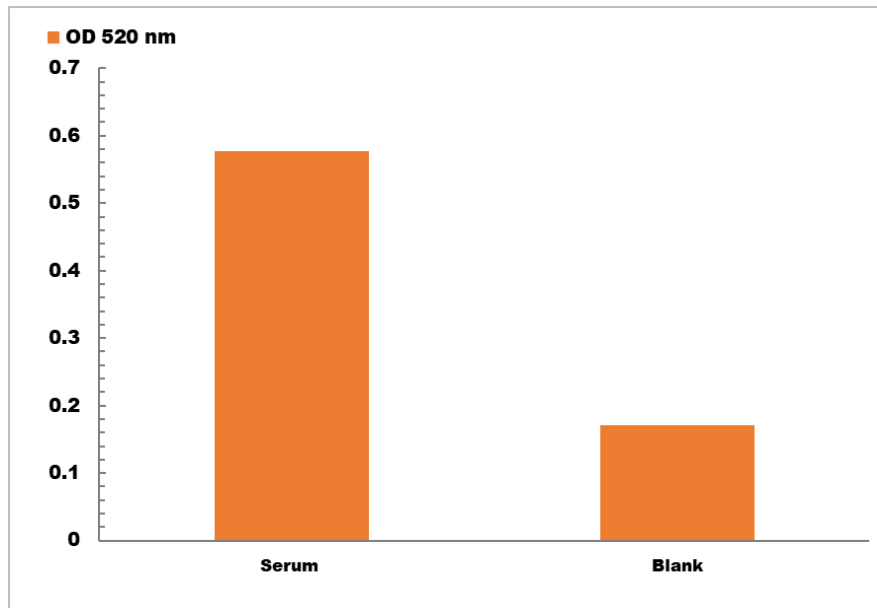
The standard curve is for demonstration only. A standard curve must be run with each assay.



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



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



Determination of alanine transaminase in serum