MA-0116

Alanine Aminotransferase Activity Assay (ALT)

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Background Information:

Alanine aminotransferase, also called ALT formerly SGPT (serum glutamic pyruvic transaminase), is found in serum and several tissues, primarily the liver. Its purpose is to shuttle the amine function between the α-ketoglutarate/alanine pair and the glutamate/pyruvate pair. A variety of disease states cause elevated ALT so it is commonly measured, along with other enzyme tests as part of a differential diagnostic test. In AkrivisBio's ALT Assay, alanine is converted to pyruvate which is oxidized forming hydrogen peroxide which is utilized by peroxidase to oxidize ADMP forming intense color (λmax, 570 nm) and fluorescence, (Excitation/Emission, 535/587 nm). AkrivisBio provides a simple sensitive assay of ALT with a detection limit below 10 mU per well.

Assay Principle:

1 – The amino group of alanine is transferred to ketoglutarate by ALT, forming pyruvate and glutamate.
2 – Pyruvate is oxidized forming hydrogen peroxide
3 – Hydrogen peroxide is utilized to convert ADHP to resorufin, resulting in intense color and fluorescence.

Assay Components:

Assay Buffer	25 ml	WM	MA-0116A
ADHP Solution	200 µl	Red	MA-0116B
Pyruvate Oxidase/HRP	lyoph	Green	MA-0116C
Alanine/α-ketoglutarate	lyoph	Orange	MA-0116D
Pyruvate Standard	100 µl	Yellow	MA-0116E
ALT Positive Control	lyoph	Blue	MA-0116F

Storage and Handling:

Store the unopened kit at -20°C. Allow all components to come to room temperature before use. Centrifuge all vials briefly before opening.
Assay Buffer: Ready to use as supplied. Store at 4°C.
ADHP Solution: DMSO freezes just below room temperature. It must be brought to room temperature before use. Store at -20°C.
Pyruvate Oxidase/HRP: Reconstitute with 220 µl DI H_2O. Aliquot into convenient portions and store at -20°C.
Alanine/α-ketoglutarate: Reconstitute with 1.1 ml Assay Buffer. Aliquot into convenient portions and store at -20°C.
Pyruvate Standard: Ready to use as supplied. Store at -20°C.
ALT Positive Control: Reconstitute with 100 µl DI H_2O. Aliquot into convenient portions and store at -20°C.

Assay Protocol:

1. Standard Curve:
Absorbance based assay: Add 10 µl of the Pyruvate Standard to 990 µl of Assay Buffer. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of the diluted standard to a series of wells in a 96-well plate. Adjust all well volumes to 25 µl with Assay Buffer giving 0 – 2 – 4 – 6 – 8 – 10 nmols per well.
Fluorescence based assay: Dilute the Pyruvate Standard as described for the absorbance-based assay. Add 10 µl of the diluted standard to 90 µl of Assay Buffer.Transfer 0–5–10–15–20–25 µl to a series of wells of a 96-well plate. Adjust all well volumes to 25 µl with Assay Buffer to generate 0-200-400-600-800-1000 pmoles per well.

2. Sample Preparation: Homogenize 10 mg tissue or 1 x 10^6 cells in 100 - 200 µl ice-cold Assay Buffer. Centrifuge at 16,000 X g, 10 min to remove cell debris. Carefully transfer the clear supernatant to a fresh tube. Serum can be used directly after dilution with Assay Buffer. Transfer test samples (5 - 20 µl) to wells in a 96-well plate. For a more precise assay use two different amounts of sample (5 and 10 µl or 10 and 20 µl). Adjust all well volumes to 25 µl. Signal from samples must fall within the range of the standard curve. If a sample is outside of this range, dilute accordingly and repeat the assay.

3. Positive Control (Optional): Transfer 2 - 5 µl to wells and adjust the well volumes to 25 µl.

4. Initiate Reaction: Each well will require 100 µl of Reaction Mix. Prepare sufficient Reaction Mix for all samples and standards.

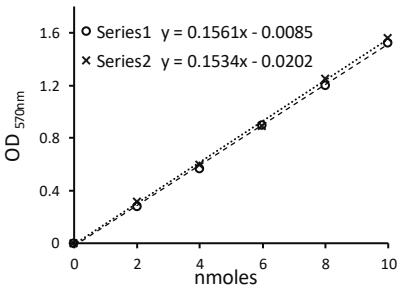
Reaction Mix:	**Absorbance based assay**	**Fluorescence based assay**
Assay Buffer	86 µl	87.6 µl
ADHP Solution	2 µl	0.4 µl
Pyruvate Oxidase/HRP	2 µl	2 µl
Alanine/α-ketoglutarate	10 µl	10 µl

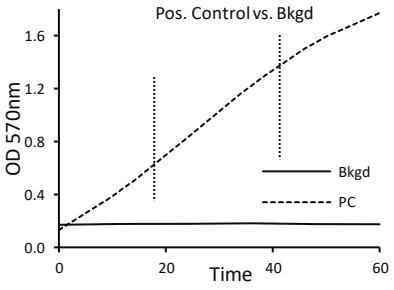
Add 100 µl of Reaction Mix to all wells (Samples, Standards, any optional Positive Control).

5. Measurement: Monitor the reaction in kinetic mode at 37°C for 30 min - 1 hour, using either absorbance or fluorescence. Use only the linear portion of the reaction signal. There will be a lag phase which can last 15-20 minutes before the reaction stabilizes at a linear rate. As the substrate is depleted, the reaction will slow. The linear range between those two phases is the data to be used to determine enzyme activity. If any sample exceeds the absorbance or fluorescence observed for the highest standard, it should be diluted and rerun. If using two different amounts of sample, the rates should be proportional to the amount used (i.e., 10 µl rate = 2X 5 µl rate)

6. Typical Results:

nmole Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0633	0.0541	0	0
2	0.3425	0.3719	0.2792	0.3178
4	0.6342	0.6558	0.5709	0.6017
6	0.9637	0.9482	0.9004	0.8941
8	1.2667	1.3059	1.2034	1.2518
10	1.5905	1.6205	1.5272	1.5664





7. Calculation: Subtract the value of the zero standard from all standard values. Plot the Standard Curve. Determine the slope of the Standard Curve. This defines the OD/nmole obtained from the experiment. Determine the rate of enzyme activity (Δ OD/ Δ minutes). Divide the rate by the slope of the standard curve to get enzyme rate in nmoles/minute (mU)in the well. To convert back to activity in the original sample:
Enzyme rate in well X (total volume of extract (from step **2.** above) / volume added to well) = Total Activity from Sample
Total Activity from Sample / mg of raw sample (µl of fluid sample before dilution) = activity as (mU)/mg or µl of sample

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