APExBIO
An Apoptosis and Epigenetics Company

Product Information

Alanine Aminotransferase (ALT or SGPT) Activity Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2170-100 100 assays	Cap Color	Part Number
ALT Assay Buffer	25 ml	WM	K2170-C-1
OxiRed™ (in DMSO)	200 µl	Red	K2170-C-2
ALT Enzyme Mix (lyophilized)	1 vial	Green	K2170-C-3
ALT Substrate (lyophilized)	1 vial	Orange	K2170-C-4
Pyruvate Standard (100 nmol/µl)	100 µl	Yellow	K2170-C-5
ALT Positive Control (lyophilized)	1 vial	Blue	K2170-C-6

II. Introduction:

Alanine aminotransferase (ALT), also known as serum glutamic pyruvic transaminase (SGPT) or alanine transaminase (ALAT) is a transaminase enzyme. ALT can be found in serum and different body tissues and is mainly found in liver. It catalyzes the reaction: α-ketoglutarate + alanine ⇌ glutamate + pyruvate. It is commonly used as a clinical test for liver health. In Alanine Aminotransferase (ALT or SGPT) Activity Colorimetric/Fluorometric Assay Kit, ALT catalyzes the transfer of an amino group from alanine to α-ketoglutarate and produce pyruvate and glutamate. The pyruvate can be detected in a reaction that concomitantly transfrom a nearly colorless probe to both color (λmax = 570 nm) and fluorescence (Ex/Em = 535/587 nm). This kit offers a reliable and sensitive way for high throughput activity assay of ALT with a detection limit of 0.05 mU per well.

III. Storage and Handling:

Store the kit at -20℃, protect from light. Allow ALT Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent preparation:

ALT Enzyme Mix: Reconstitute with 220 µl dH_2O. Aliquot and store at -20℃. Use within two months.

ALT Substrate: Reconstitute with 1.1 ml Assay Buffer. Aliquot and store at -20℃. Use within two months.

ALT Positive Control: Reconstitute with 100 µl dH_2O. Aliquot and store at -20℃, use within two months. In the assay (optional), add 5 - 10 µl positive control and adjust the final volume to 20 µl/well with ALT Assay Buffer.

V. ALT Assay Protocol:

1. Standard Curve Preparation:

Colorimetric assay: Dilute the Pyruvate Standard to 1 nmol/µl by adding 10 µl of the Standard to 990 µl of ALT Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of standards wells. Adjust volume to 20 µl/well with ALT Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Pyruvate Standard for the colorimetric assay.

Fluorometric assay: Dilute the Pyruvate Standard to 1 nmol/µl as for the colorimetric assay. Then dilute the standard another 10-fold to 0.1 nmol/µl by taking 10 µl into 90 µl of ALT Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of standards wells. Adjust volume to 20 µl/well with ALT Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Pyruvate Standard for the fluorometric assay.

2. Sample Preparations: Tissues (50 mg) or cells (1 x 10^6) can be homogenized in 200 µl icecold ALT Assay Buffer, then centrifuged (13,000 x g, 10 min) to remove insoluble material.

3. Serum samples can be directly diluted in the Assay Buffer. Prepare test samples of up to 20 µl/well with Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.

4. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 100 µl Reaction Mix:

ALT Assay Buffer	86 µl
OxiRed Probe	2 µl
ALT Enzyme Mix	2 µl
ALT Substrate	10 µl

Add 100 µl of the Sample Reaction Mix to each well containing the Samples, Standards, and Positive Controls (optional). Mix well.

Note: The fluorometric assay is ~ 10 times more sensitive than the colorimetric assay. Use 0.4 µl of the probe per reaction to decrease the background reading & increase detection sensitivity significantly.

5. Measurement: Read OD 570 nm (A1) at T1 (T1 > 10min) then again (A2) at T2 after incubating the reaction at 37℃ for 60 min (or longer if the ALT activity is low), protect from light. The OD of the color generated by oxidation of pyruvate is ΔA570 nm = A2 – A1. It is recommended that the user run the assay kinetically to choose A1 and A2 values which occur after the initial lag phase, during the linear range of color development. OD at A2 should not exceed the highest OD in the standard curve.

6. Calculation: Plot the pyruvate Standard Curve and use the ΔA570 nm to obtain B nmol of pyruvate (amount of pyruvate generated between T1 and T2 in the reaction wells). ALT activity in the test samples can then be calculated:

$$\text{ALT Activity} = B / [(T2 - T1) \times V] = \text{nmol/min/ml} = \text{mU/ml}$$

Where: B is the pyruvate amount from pyruvate Standard Curve (in nmol).

T1 is the time of the first reading (A1) (in min).

T2 is the time of the second reading (A2) (in min).

V is the original sample volume added into the reaction well (in ml).

One unit of ALT is defined as the amount of ALT which generates 1.0 µmol of pyruvate per minute at 37℃.

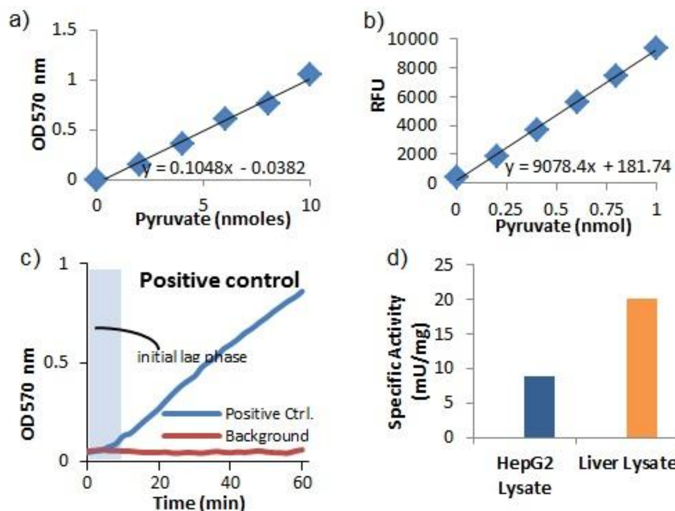

Figure: Pyruvate Standard Curve a) Colorimetric, b) Fluorometric. Measurement of alanine aminotransferase activity in Positive Control (c) and HepG2 Cells (10 ug) and Liver Lysate (15 ug) (d). Assays were performed following the kit protocol.

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General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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