ab105134 Alanine Transaminase Activity Assay kit (Colorimetric/Fluorometric)

For the rapid, sensitive and accurate measurement of Alanine Transaminase (ALT) activity in various mammalian samples.

View kit datasheet: www.abcam.com/ab105134

(use www.abcam.cn/ab105134 for China, or www.abcam.co.jp/ab105134 for Japan)

This product is for research use only and is not intended for diagnostic use

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stability: Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
Aliquot components in working volumes before storing at the recommended temperature. Reconstituted components are stable for 2 months.

Materials Supplied:

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer XIII/ALT Assay Buffer	25 mL	-20°C	-20°C
OxiRed Probe/OxiRed Probe (in DMSO)	200 µL	-20°C	-20°C
Development Enzyme Mix I/ALT Enzyme Mix (Lyophilized)	1 vial	-20°C	-20°C
ALT Substrate Mix/ALT Substrate (Lyophilized)	1 vial	-20°C	-20°C
Pyruvate Standard/Pyruvate Standard (100 nmol/µlL	100 µL	-20°C	-20°C
ALT Positive Control/ALT Positive Control (Lyophilized)	1 vial	-20°C	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH2O)
- Cold PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader – equipped with filter for OD570 nm or Ex/Em = 535/587 nm (respectively)
- 96 well plate: clear plates for colorimetric assay; black plates (clear bottoms) for fluorometric assay
- Orbital shaker
- Heat bock or water bath
- Vortex
- Dounce homogenizer or pestle (if using tissue)

Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

ALT Assay Buffer: Ready to use. Equilibrate to room temperature before use. Store at -20°C.

OxiRed Probe: Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use.

NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.

Aliquot probe so that you have enough volume to perform the desired number of tests. Store at -20°C protected from light. Once the probe is thawed, use with two months.

Development Enzyme Mix I/ALT Enzyme Mix: Reconstitute with 220 µL ddH_2O. Aliquot enzyme mix so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within two months. Keep on ice while in use.

ALT Substrate: Reconstitute with 440 µL of ddH_2O. Aliquot substrate so that you have enough volume to perform the desired number of tests. Store at -20°C. Keep on ice while in use.

Pyruvate Standard: Ready to use as supplied. Aliquot standard so that you have enough to perform the desired number of tests. Store at -20°C. Keep on ice while in use.

ALT Positive Control: Reconstitute with 100 µL ddH_2O. Aliquot positive control so that you have enough volume to perform the desired number of tests. Store at -20°C. Keep on ice while in use.

Standard Preparation

Always prepare a fresh set of standards for every use.

Diluted standard solution is unstable and must be used within 4 hours.

For the colorimetric Assay

1. Prepare a 1 nmol/µL Pyruvate Standard by diluting 5 µL Pyruvate Standard in 495 µL of ALT Assay Buffer.
2. Using 1 nmol/µL standard, prepare standard curve dilution as described in the table below in a microplate or microcentrifuge tubes

For the Fluorometric Assay

1. Prepare a 1 nmol/µL Pyruvate Standard by diluting 5 µL Pyruvate Standard in 495 µL of ALT Assay Buffer
2. Prepare a 0.1 nmol/µL Pyruvate Standard by diluting 100 µL 1nmol/µL Pyruvate Standard in 900 µL of ALT Assay Buffer.
3. Using 0.1 nmol/µL Pyruvate Standard, prepare standard curve dilution as described in the table below in a microplate or microcentrifuge tube

Standard #	Volume of standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End [alanine transaminase] in well	
				Colorimetric assay	Fluorometric assay
1	0	60	20	0 nmol/well	0 nmol/well
2	6	54	20	2 nmol/well	0.2 nmol/well
3	12	48	20	4 nmol/well	0.4 nmol/well
4	18	42	20	6 nmol/well	0.6 nmol/well
5	24	36	20	8 nmol/well	0.8 nmol/well
6	30	30	20	10 nmol/well	1.0 nmol/well

Each dilution has enough amount of standard to set up duplicate reading (2 x 20 µL).

Sample preparation

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

Cell (adherent or suspension) samples:

1. Harvest amount of cells necessary for each assay (initial recommendation = 1 x 106 cells).
2. Wash cells with cold PBS.
3. Resuspend cells in 200 µL ice cold ALT Assay Buffer.
4. Homogenize cells quickly by pipetting up and down a few times.
5. Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube and keep on ice.

Tissue samples:

1. Harvest the amount of tissue necessary for each assay (initial recommendation = 50 mg tissue).
2. Wash tissue in cold PBS.
3. Resuspend tissue in ~ 200 µL ice cold ALT Assay Buffer.
4. Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
5. Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube and keep on ice.

Serum and urine samples:

- Serum samples can be directly can be tested directly by adding sample to the microplate wells.
- However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (1/2 – 1/5 – 1/10).

NOTE: We suggest using different volumes of sample to ensure readings are within the Standard Curve range.

Assay procedure and detection

Equilibrate all materials and prepared reagents to room temperature prior to use.
It is recommended to assay all standards, controls and samples in duplicate.

1. **Set up Reaction wells:**

- Standard wells = 20 µL Standard dilutions.
- Sample wells = 2 – 20 µL samples (adjust volume to 20 µL/well with ALT Assay Buffer).
- (OPTIONAL) Positive control = 2 - 4 µL Positive control (adjust volume to 20 µL/well with ALT Buffer).

2. **Reaction Mix:**

- Prepare 100 µL of Reaction Mix for each reaction:

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)
ALT Assay Buffer	92	93.6
OxiRed Probe*	2	0.4
Development Enzyme Mix I/ALT Enzyme Mix	2	2
ALT Substrate	4	4

**NOTE: ***For fluorometric reading, using 0.4µL/well of the OxiRed probe decreases the background readings, therefore increasing detection sensitivity.
Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:
X µL component x (Number of samples + standards +1).

3. **Add 100 µL of Reaction Mix into each well of standard, samples and positive controls if using.**
4. **Measure output on a microplate reader in a kinetic mode after 10 minutes, every 2 – 3 minutes**, for at least 60 minutes at 37°C protected from light.

- Colorimetric assay: measure OD570 nm.
- Fluorometric assay: measure Ex/Em = 535/587nm.
- There is an initial lag phase of 10 minutes that can lead to underestimation of the ALT activity. We recommend measuring activity after the initial 10 min incubation.

NOTE: Sample incubation time can vary depending on ALT activity in the samples. We recommend measuring absorbance/fluorescence in kinetic mode and then choosing two time points (T_1 and T_2) after the initial lag phase, during the linear range.
RFU/OD value at T_2 should not exceed the highest OD in the standard curve. For standard curve, do not subtract A_1 from A_2 reading.

Calculations

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
We recommend each sample should be assayed with a minimum of two replicates (duplicates).

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of the final concentration of Pyruvate.
4. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
5. Activity of ALT is calculated as:
6. ΔA570nm / ΔRFU535/575nm = A2 – A1
7. Where:
8. A1 is the sample reading at time T1
9. A2 is the sample reading at time T2
10. Use the ΔA570nm/ ΔRFU535/575nm to obtain B nmol of Pyruvate generated by ALT during the reaction time (ΔT = T2 – T1).
11. Concentration of pyruvate in the test samples is calculated as:

$$\text{ALT Activity} = \left(\frac{\text{B}}{\Delta\text{T} \times \text{V}}\right) * \text{D}$$

ALT activity= nmol/min/mL =mU/mL

Where:
B = Amount of pyruvate from Pyruvate Standard Curve.
ΔT = reaction time (min).
V = original sample volume added into the reaction well (in mL).
D = sample dilution factor.
ALT molecular weight: 54.47 g/mol.

Unit Definition:

1 Unit ALT = amount of ALT which generates 1.0 µmol of Pyruvate per min at 37°C.
For example, if you added 10 µl of undiluted cell lysate and make up the volume in the 96 well up to 50 µl using assay buffer, your V is 0.01 and dilution factor is 1. Alternatively, if you added 10 µl of (1:10 diluted cell lysate) and make up the volume in the 96 well up to 50 µl using assay buffer, your V is 0.01 and dilution factor is 10.

NOTE: We recommend measuring the protein concentration of the homogenized samples when using cells or tissue to normalize the results by mg of protein.

FAQ

At what time points should the standards and samples be read?
OD measurement must be done at an initial time point T1 (T1 > 10min) then again at T2 after incubating the reaction at 37 °C for 60 min (or longer if the ALT activity is low). T2 should be chosen such that the OD at this time T2 is within the OD for the highest standard. The exact time points will need to be optimized based on the level of activity in your samples. These times provided on the datasheet are only guidelines. The standards are read at endpoint (highest timepoint for the samples).

Why can't this assay be done at endpoint only?
Enzyme activity is a rate of change with respect to time. Enzyme activity varies with time and hence to calculate rate accurately, change in OD over a time difference is to be measured. Doing an endpoint assay is only a rough estimate. The data should be analyzed within the linear increase regime of a Michaelis-Menten curve.

Why does T1 have to be > 10 minutes?
The 2 – 3 minute measurement is within the lag phase as is shown by the grey shaded area on the positive control data plot (see figure 3). Taking a measurement within this time is not recommended and can definitely result in major underestimation of the ALT activity. We recommend making the initial measurement at 10 minutes and no earlier than that to avoid being in the lag phase.

The kit recommends adding up to 20 µL of sample to 100 µL of mastermix, however we are having trouble with our samples having low levels of ALT. Would it be possible to add a greater amount of sample to mastermix e.g. 50 µL of sample to 100 µL?
If there is less ALT in your sample, we suggest you run the fluorometric version of the assay using 20 µL sample but lowering the probe to 0.4 µL. The fluorometric method is 10 – 100 times more sensitive and should work for any samples with low ALT activity.

Can this kit work with rat or pig samples?
Our kits can work with a variety of mammalian samples including rat and pig. You might have to try a few different volumes to make sure the readings are within the linear range of the std. curve. This kit has a detection limit of 10 mU of enzyme activity per well.

Can ALT be measured from serum or cell culture media? What type of cells are used normally to test ALT activity?
Typically liver cells or liver-derived cell lines (HepG2 cells, for example) are commonly used. Liver cells release these enzymes when there is damage to the cells and hence in such situations can be measured in the culture medium or serum. Normally, levels in the serum is low. The normal range of values for ALT (SGPT) ranges from 7 – 56 units per liter of human serum.

Can RIPA buffer be used to prepare samples for this kit?
For any enzyme assay, we do not recommend RIPA buffer since it contains SDS and this can denature proteins and affect enzyme activity. We have tested and recommend using our assay buffer provided in the kit for best results.

Interferences

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- RIPA buffer – contains SDS which can denature proteins and affect enzyme activity.

Troubleshooting

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol

Technical Support

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