Version v4a Last updated 23 June 2025

ab241035
ALT Assay Kit (384 well, Colorimetric/Fluorometric)

For the mearurement of alanine aminotransferase activity in various biological samples.

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

Table of Contents

1. Overview

The ALT Assay Kit (384 well, Colorimetric/Fluorometric) (ab241035) provides a rapid, sensitive and reliable test, suitable for high throughput screening of ALT activity in various biological samples.

The 384-well format allows for the screening of a large number of samples on a single high-density microplate. The kit can be run in either colorimetric or fluorometric detection mode and can detect a minimum of 0.25 to 1.25 mU of ALT activity per well, using a minimal sample volume as low as 0.5 µl.

2. Protocol Summary

Prepare all samples, controls and standards as instructed.

Prepare the Pyruvate standard curve for the colorimetric and fluorometric assay.

Add 0.5-2.5 µL of sample to desired wells, adjust volume to 5 µL with ALT Assay Buffer.

Prepare the reaction mix and add 25 µL to each well containing samples, standards and positive controls.

Add 25 µL of background Mix to each well containing the background test samples.

Measure the absorbance (OD_{570}) or fluorescence (Ex/Em = 535/587 nm) in kinetic mode for 60 min or longer at 37°C.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
Assay Buffer 13	25 mL	-20°C
OxiRed™ Probe	0.2 mL	-20°C
Developer Mix A	1 vial	-20°C
ALT Substrate Mix	1 vial	-20°C
Pyruvate Standard	100 µL	-20°C
ALT Positive control	1 vial	-20°C

PLEASE NOTE: Assay Buffer 13 was previously labelled as Assay Buffer XIII and ALT Assay Buffer, and Developer Mix A as Development Enzyme Mix I and ALT Enzyme Mix. OxiRed™ Probe was previously labelled as OxiRed Probe and Red Probe. The composition has not changed.

5. Materials Required, Not Supplied

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

- 384-well clear plate with flat bottom for colorimetric assay; black or clear 384-well plate for fluorometric assay.
- Multi-well spectrophotometer

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 Assay Buffer 13:

Ready to use as supplied. Bring to room temperature (RT) before use.

6.2 OxiRed™ Probe:

Ready to use as supplied. Warm to room temperature to thaw the probe solution prior to use. Store at -20°C, protect from light. Use within two months.

6.3 Developer Mix A:

Reconstitute in 220 µl ddH2O. Mix gently but thoroughly, aliquot as desired and store at -20°C. Prior to use, allow to thaw at room temperature for several minutes, then gently mix and place on ice. Keep on ice while in use. Use within two months.

6.4 ALT Substrate Mix:

Reconstitute in 1.1 mL of ALT Assay Buffer and mix thoroughly. Aliquot as desired and store at -20°C. Keep on ice while in use. Use reconstituted stock within two months.

6.5 ALT Positive control:

Reconstitute in 100 µl of ddH_2O, aliquot as desired and store at -20°C. Keep on ice while in use. Use within two months.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 **Colorimetric Assay:** Dilute the Pyruvate Standard (100 mM) stock solution to 0.5 nmol/µl by mixing 5 µl of the Standard with 995 µl of Assay Buffer 13.

7.2 Add 0, 1, 2, 3, 4, 5 µl into a series of standard wells on a 384-well plate.

7.3 Adjust the volume to 5 µl/well with Assay Buffer 13 to generate 0, 0.5, 1.0, 1.5, 2, and 2.5 nmol/well of Pyruvate Standard for the colorimetric assay.

Standard #	0.5 nmol/µL Standard (µL)	Assay Buffer 13 (µL)	Pyruvate standard nmol/well
1	5	0	2.5
2	4	1	2
3	3	2	1.5
4	2	3	1
5	1	4	0.5
6	0	5	0

7.4 **Fluorometric Assay:** Dilute the Pyruvate Standard (100 mM) stock solution to 1 nmol/µl by mixing 10 µl of the Standard with 990 µl of Assay Buffer 13.

7.5 Further dilute the Standard another 10-fold to 0.1 nmol/ µl by mixing 10 µl of the 1 nmol/µl solution with 90 µl of Assay Buffer 13.

7.6 Add 0, 0.5, 1, 1.5, 2, 2.5 µl into a series of standard wells on a 384-well plate. Adjust the volume to 5 µl/well with Assay Buffer 13 to generate 0, 0.05, 0.1, 0.15, 0.2, and 0.25 nmol/well of Pyruvate Standard for the fluorometric assay.

Standard #	**0.1 nmol/µL Standard (µL)**	**Assay Buffer 13 (µL)**	**Pyruvate standard nmol/well**
1	2.5	2.5	0.25
2	2	3	0.2
3	1.5	3.5	0.15
4	1	4	0.1
5	0.5	4.5	0.05
6	0	5	0

8. Sample Preparation

- As ALT activity levels can vary dramatically between samples, we suggest testing different volumes of your sample to ensure that the readings are within the standard curve range. Samples with extremely high ALT activity may be diluted with ALT Assay Buffer.
- For samples having background, prepare parallel background well(s) containing same amount of sample as in the test well.

8.1 Homogenate samples:

- Tissues (~50 mg wet tissue) or pelleted cells (~106 cells) can be homogenized in ~200 µl of ice-cold Assay Buffer 13, then centrifuged (13,000 x g) at 4°C for 10 min to remove any insoluble material or cellular debris.
- Following centrifugation, transfer supernatant to a fresh microfuge tube and keep on ice during use. Add 0.5 - 2.5 µl of sample homogenate per well and adjust the volume to 5 µl/well with Assay Buffer 13.

8.2 Serum:

- Samples can be run directly without prior sample preparation. For serum samples, add 0.5 - 2.5 µl of serum per well and adjust the volume to 5 µl/well with Assay Buffer 13.

8.3 Positive control:

- Prepare positive control wells by adding 2 µl of the reconstituted ALT Positive Control stock solution to the well(s) and adjusting the volume to 5 µl/well with Assay Buffer 13.

9. Assay Procedure

9.1 Prepare enough Reaction Mix for the number of assays to be performed (including Pyruvate Standard curve and Positive Control wells).

9.2 For each well, prepare 25.0 µl Reaction Mix containing:

	Colorimetric	**Background**	**Flurometric**	**Background**
Assay Buffer 13	21.5 µL	24 µL	21.9 µL	24.4 µL
OxiRed™ Probe	0.5 µL	0.5 µL	0.1 µL	0.1 µL
Developer Mix A	0.5 µL	0.5 µL	0.5 µL	0.5 µL
ALT Substrate Mix	2.5 µL	/	2.5 µL	/

9.3 Add 25 µl of Reaction Mix to each well containing the test samples, Pyruvate Standards, or ALT Positive Control.

9.4 For sample background control wells, add 25 µl of the Sample Background reaction mix.

∆**Note:** *The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Using 0.1 µl of the probe per reaction decreases the background reading and increases detection sensitivity significantly.*

9.5 **Measurement:** Measure the absorbance (OD570) or fluorescence (Ex/Em = 535/587 nm) in kinetic mode for 60 min or longer at 37°C**.**

∆**Note:** *While the assay can be performed in either end-point or kinetic mode, we strongly recommend reading in kinetic mode in*

order to ensure that the measurements recorded are within the linear range of the reaction.

10. Data Analysis

10.1 Subtract the zero L-Threonine Standard curve from all Standard readings. Plot the Standard Curve.

10.2 For each reaction well (sample, standard, background control, and positive control wells), choose two time points (t_1 and t_2) in the linear phase of the reaction progress curves, and obtain the corresponding absorbance (A_1 and A_2) or fluorescence (RFU_1 and RFU_2) values at those time points and determine the change in absorbance or fluorescence signal over the time interval: $\Delta OD_{570} = A_2 – A_1$ or $\Delta F = RFU_2 – RFU_1$. Subtract the 0 nmol-standard ΔOD_{570} or ΔF value from the rest of the standards' ΔOD_{570} or ΔF values. Plot all standards' ΔOD_{570} or ΔF values against their corresponding nmol Pyruvate; this should trace out a linear relationship between standard nmol Pyruvate and ΔOD_{570} or ΔF. Use linear regression to model this relationship for interpolation of the sample's ΔOD_{570} or ΔF values. Subtract positive background control ΔOD_{570} or ΔF value from its corresponding sample's ΔOD_{570} or ΔF value; if the background values are negative, ignore them.

∆**Note:** *Choose time points which occur after the initial lag phase (roughly 5-10 min in our experience) and during the linear range of probe development (usually within 60 min, samples with extremely low ALT activity may require longer). For Standards, choose only one time point's values and subtract the 0 Standard value from all standard values from that timepoint. Use the background-subtracted standard values to plot the standard curve.*

10.3 The ΔOD_{570} or ΔF value should fall within the range of the Pyruvate Standard curve.

∆**Note:** *Microplate reader settings may need to be adjusted according to the chosen 384-well plate. The dimensions of the used*

384-well plate may be available in the manual provided by the plate manufacturer.

10.4 **Calculation** apply the sample ΔOD_{570} or ΔF values to the standard curve to obtain B nmol of pyruvate (the amount generated between times t_1 and t_2) in the sample well. ALT activity in test samples can be then be calculated:

$$\textbf{Sample ALT Activity} = \frac{\boldsymbol{B}}{(\boldsymbol{t_2}-\boldsymbol{t_1}) \times \boldsymbol{V}} = \textbf{nmol/min/mL} = \textbf{mU/mL}$$

Where:

B is the amount of pyruvate, in the sample well from Standard Curve (nmol)

t1 is the time of the first reading (in min)

t2 is the time of the second reading (in min)

V is original volume added into the reaction well (mL)

Δ Note: *One unit is defined as the amount of ALT which generates 1.0 µmol of pyruvate per minute at 37°C.*

11. Typical Data

Typical data provided **for demonstration purposes only**.

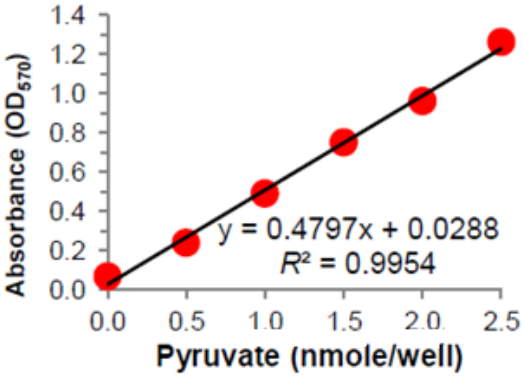

Figure 1. Pyruvate Standard Curve (colorimetric)

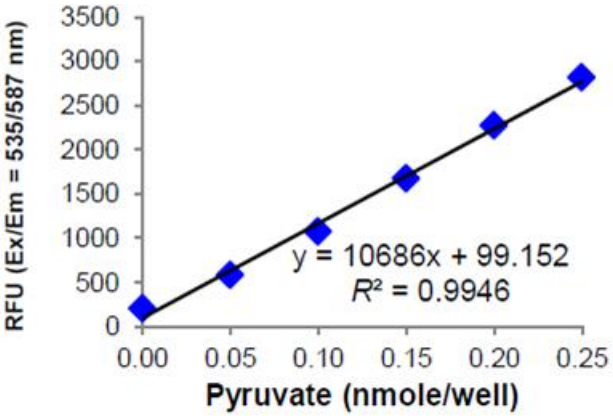

Figure 2. Pyruvate standard curve (Fluorometric)

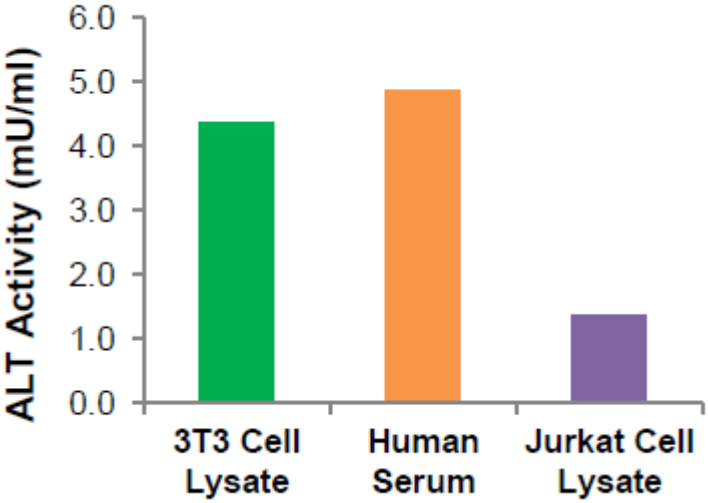

Figure 3. ALT Activity (mU/ml) in 3T3 Cell Lysate (2.5 µl, 12 mg/ml protein). Pooled Human Serum (2.5 µl), and Jurkat Cell Lysate (1.25 µl, 4 mg/ml protein). Assays were performed according to the kit protocol.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)