

Version v4b Last updated 16 July 2026

# **ab241035**

## **ALT Assay Kit (384 well, Colorimetric/Fluorometric)**

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

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

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# 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  $\mu$ l.

## 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  $\mu\text{L}$  of sample to desired wells, adjust volume to 5  $\mu\text{L}$  with ALT Assay Buffer.



Prepare the reaction mix and add 25  $\mu\text{L}$  to each well containing samples, standards and positive controls.



Add 25  $\mu\text{L}$  of background Mix to each well containing the background test samples.



Measure the absorbance ( $\text{OD}_{570}$ ) or fluorescence ( $\text{Ex/Em} = 535/587 \text{ nm}$ ) in kinetic mode for 60 min or longer at  $37^\circ\text{C}$ .

### 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](http://www.abcam.com/assaykitguidelines)
- For typical data produced using the assay, please see the assay kit datasheet on our website.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website <https://www.abcam.com/en-us>.

## 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.
- 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 the DMSO solution at 37 °C until completely thawed (~ 5 minutes), vortex briefly to create a homogenous solution, and spin down briefly to collect any contents trapped in the cap or seal. Keep at room temperature during use and reseal promptly to minimize moisture exposure.

For multiple use aliquot and store at -20 °C, protected from light and moisture. Stable for up to 2 months after thawing (≤3 freeze-thaw cycles).

### 6.3 Developer Mix A:

Reconstitute in 220 µl ddH<sub>2</sub>O. 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<sub>2</sub>O, 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/ $\mu$ L by mixing 5  $\mu$ L of the Standard with 995  $\mu$ L of Assay Buffer 13.
- 7.2** Add 0, 1, 2, 3, 4, 5  $\mu$ L into a series of standard wells on a 384-well plate.
- 7.3** Adjust the volume to 5  $\mu$ L/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/ $\mu$ L Standard ( $\mu$ L) | Assay Buffer 13 ( $\mu$ L) | 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/ $\mu$ L by mixing 10  $\mu$ L of the Standard with 990  $\mu$ L of Assay Buffer 13.
- 7.5** Further dilute the Standard another 10-fold to 0.1 nmol/ $\mu$ L by mixing 10  $\mu$ L of the 1 nmol/ $\mu$ L solution with 90  $\mu$ L of Assay Buffer 13.
- 7.6** Add 0, 0.5, 1, 1.5, 2, 2.5  $\mu$ L into a series of standard wells on a 384-well plate. Adjust the volume to 5  $\mu$ L/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/ $\mu$ L Standard ( $\mu$ L) | Assay Buffer 13 ( $\mu$ L) | 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 (~10<sup>6</sup> 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 | Fluorometric | Background |
|--------------------------|--------------|------------|--------------|------------|
| <b>Assay Buffer 13</b>   | 21.5 µL      | 24 µL      | 21.9 µL      | 24.4 µL    |
| <b>OxiRed™ Probe</b>     | 0.5 µL       | 0.5 µL     | 0.1 µL       | 0.1 µL     |
| <b>Developer Mix A</b>   | 0.5 µL       | 0.5 µL     | 0.5 µL       | 0.5 µL     |
| <b>ALT Substrate Mix</b> | 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.

**ΔNote:** 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.

**ΔNote:** 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<sub>1</sub> and RFU<sub>2</sub>) 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  $\Delta OD_{570}$  or  $\Delta F$  value from the rest of the standards'  $\Delta OD_{570}$  or  $\Delta F$  values. Plot all standards'  $\Delta OD_{570}$  or  $\Delta F$  values against their corresponding nmol Pyruvate; this should trace out a linear relationship between standard nmol Pyruvate and  $\Delta OD_{570}$  or  $\Delta F$ . Use linear regression to model this relationship for interpolation of the sample's  $\Delta OD_{570}$  or  $\Delta F$  values. Subtract positive background control  $\Delta OD_{570}$  or  $\Delta F$  value from its corresponding sample's  $\Delta OD_{570}$  or  $\Delta F$  value; if the background values are negative, ignore them.

**ΔNote:** 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  $\Delta OD_{570}$  or  $\Delta F$  value should fall within the range of the Pyruvate Standard curve.

**ΔNote:** 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  $\Delta OD_{570}$  or  $\Delta F$  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:

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

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)

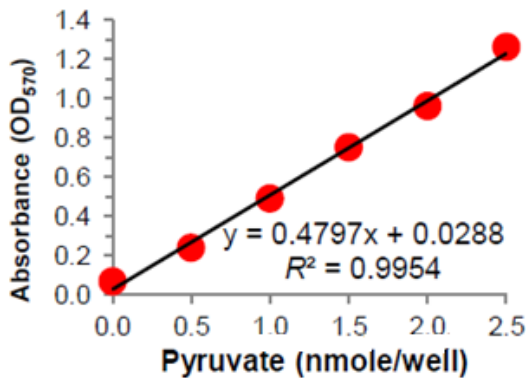
**t2** is the time of the second reading (in min)

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

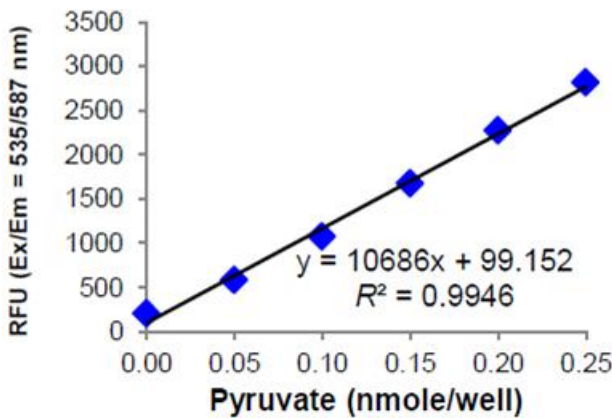
**Δ Note:** One unit is defined as the amount of ALT which generates 1.0  $\mu\text{mol}$  of pyruvate per minute at 37°C.

# 11. Typical Data

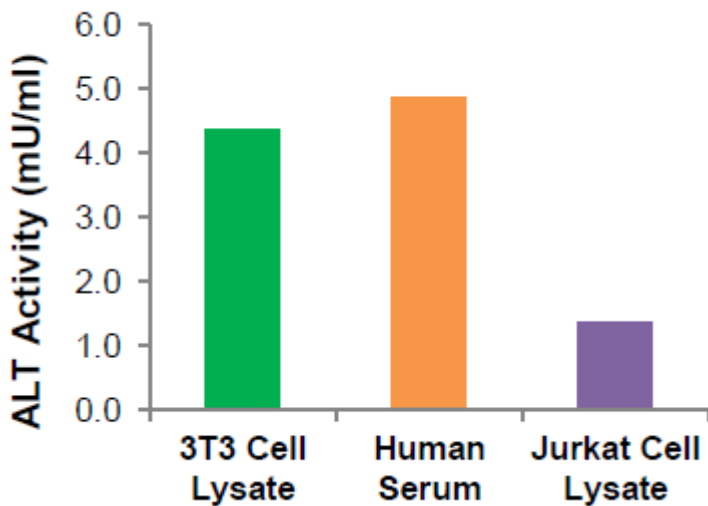
Typical data provided for demonstration purposes only.



**Figure 1.** Pyruvate Standard Curve (colorimetric)



**Figure 2.** Pyruvate standard curve (Fluorometric)



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

## 12. Notes







## **Technical Support**

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