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EZScreen™ Alanine Aminotransferase Activity Assay Kit (384-Well)

rev 04/21

(Catalog # K941-400; 400 assays; Store at -20 °C)

I. Introduction:

Alanine aminotransferase (ALT) is a transaminase enzyme (EC 2.6.1.2) formerly known as serum glutamic pyruvic transaminase (SGPT). ALT catalyzes the reversible reaction: α-ketoglutarate + alanine ⇆ glutamate + pyruvate. ALT can be found in serum and various body tissues, but is primarily expressed in the liver. Damage to hepatocytes can cause ALT to leak into systemic circulation, making serum ALT activity a useful clinical diagnostic for determination of liver health. Liver damage incurred from various infectious diseases (viral hepatitis) or hepatotoxic drugs (ethanol, acetaminophen) and may result in drastic increases in serum ALT activity. In BioVision's ALT Activity Assay Kit, ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, forming pyruvate and glutamate. Pyruvate is then detected in an enzymatic reaction that converts a nearly colorless probe into a bright chromophore/fluorophore that can be readily detected by visible absorbance spectrophotometry (λ_{max} = 570 nm) or fluorescence (Ex/Em = 535/587 nm). **BioVision's EZScreen™ ALT Assay Kit** 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.

II. Applications:

- Measurement of ALT activity in various biological samples
- Analysis of liver activity or liver injury
- High throughput screening of biological samples

III. Sample Types:

- Serum, plasma and other bodily fluids
- Cultured cell lysates of adherent or suspension cells, cell growth media
- Tissue samples

IV. Kit Contents:

Components	K941-400	Cap Code	Part Number
ALT Assay Buffer	25 ml	WM	K941-400-1
OxiRed™ Probe	0.2 ml	Red	K941-400-2
ALT Enzyme Mix	1 vial	Green	K941-400-3
ALT Substrate	1 vial	Orange	K941-400-4
Pyruvate Standard	0.1 ml	Yellow	K941-400-5
ALT Positive Control	1 vial	Blue	K941-400-6

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening. Read entire protocol before performing the assay.

- **ALT Assay Buffer:** Warm to room temperature (RT) prior to use. Store at -20 °C or 4 °C.
- **OxiRed™ Probe:** Ready to use as supplied. Warm to RT to thaw the probe solution prior to use. Store at -20 °C, protected from light. Use within two months.
- **ALT Enzyme Mix:** Reconstitute in 220 µl ddH_2O. Mix gently but thoroughly, aliquot as desired and store at -2 0°C. Prior to use, allow to thaw at RT for several minutes, then gently mix and place on ice. Keep on ice while in use. Use within two months.
- **ALT Substrate:** Reconstitute in 1.1 ml of ALT Assay Buffer and mix thoroughly. Divide into aliquots as desired and store at -20 °C. Keep on ice while in use. Use reconstituted stock within two months.
- **Pyruvate Standard:** Store at -20 °C. Keep on ice while in use.
- **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.

VII. ALT Activity Assay Protocol:

1. Sample Preparation:

Homogenate samples: Tissues (~50 mg wet tissue) or pelleted cells (~10^6 cells) can be homogenized in ~200 µl of ice-cold ALT Assay Buffer. Centrifuge at 13,000 x *g* at 4 °C for 10 min to remove any insoluble material or cellular debris. Following centrifugation, transfer the 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 ALT Assay Buffer.

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 ALT Assay Buffer.

Positive Control: Prepare positive control wells by adding 0.5-2.5 µl of the reconstituted ALT Positive Control stock solution to the well(s) and adjusting the volume to 5 µl/well with ALT Assay Buffer.

Notes: a. 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.

b. For samples having background, prepare parallel background well(s) containing same amount of sample as in the test well.

2. Standard Curve Preparation:

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 ALT Assay Buffer. Add 0, 1, 2, 3, 4, 5 µl into a series of standard wells on a 384-well plate. Adjust the volume to 5 µl/well with ALT Assay Buffer to generate 0, 0.5, 1.0, 1.5, 2, and 2.5 nmol/well of Pyruvate Standard for the colorimetric assay.

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 ALT Assay Buffer. 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 ALT Assay Buffer. 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 ALT Assay Buffer to generate 0, 0.05, 0.1, 0.15, 0.2, and 0.25 nmol/well of Pyruvate Standard for the fluorometric assay.

3. Reaction Mix: Prepare enough Reaction Mix for the number of assays to be performed (including Pyruvate Standard Curve and Positive Control wells). For each well, prepare 25.0 µl Reaction Mix containing:

	Colorimetric	**Background**	Fluorometric	Background
ALT Assay Buffer	21.5 µl	24.0 µl	21.9 µl	24.4 µl
OxiRed™ Probe	0.5 µl	0.5 µl	0.1 µl	0.1 µl
ALT Enzyme Mix	0.5 µl	0.5 µl	0.5 µl	0.5 µl
ALT Substrate	2.5 µl	---	2.5 µl	---

Add 25.0 µl of the Reaction Mix to each well containing the test samples, Pyruvate Standards, or ALT Positive Control. Add 25.0 µl of the Background Mix to each well containing the background test samples *The final volume will be 30 µl per well.*

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.

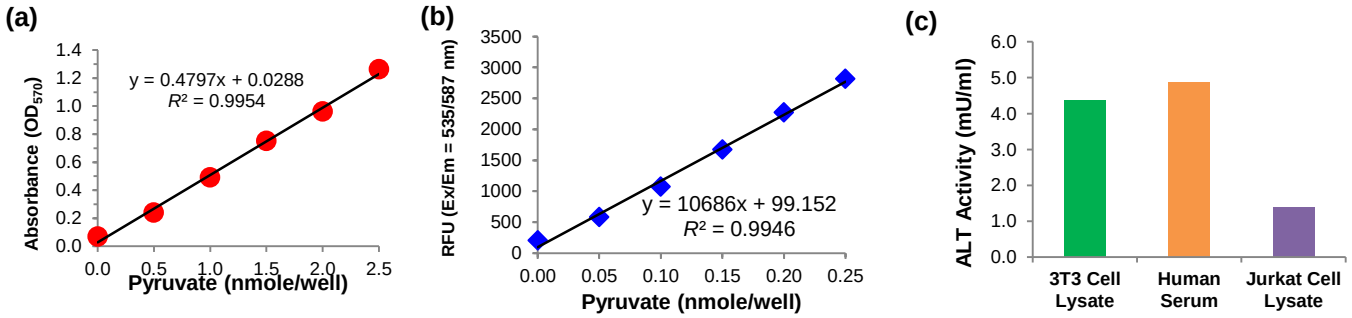
4. Measurement: Measure the absorbance ($OD_{570\ nm}$) or fluorescence (Ex/Em = 535/587 nm) in kinetic mode for 60 min or longer at 37 °C. **Notes: a.** While the assay can be performed in either end-point or kinetic mode, we strongly recommend reading in kinetic mode in order to ensure that the measurements recorded are within the linear range of the reaction. **b.** 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.

5. Calculation: Subtract the 0 Standard reading from all Standard readings and plot the Pyruvate Standard Curve. For each sample type, choose any two time points (t_1 and t_2) in the linear phase of the reaction curve 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: $\Delta OD_{570\ nm} = A_2 - A_1$ or $\Delta F = RFU_2 - RFU_1$. **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).* **If Sample Background Control reading is significant then subtract the Sample Background Control reading from its paired sample readings to get the corrected sample reading.** *A*pply the sample $\Delta OD_{570\ nm}$ or ΔF values to the Pyruvate Standard Curve to obtain *B* nmol of pyruvate generated in the sample well during the reaction time ($\Delta t = t_2 - t_1$). Calculate the ALT activity of the test samples using the following equation:

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

Where: ***B*** is the amount of pyruvate in the sample well, calculated from the Standard Curve (in nmol)
t_1 is the time of the first reading (in min)
t_2 is the time of the second reading (in min)
V is the original sample volume added into the reaction well (in ml)

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



Figures: (a) Pyruvate Standard Curve (colorimetric). **(b)** Pyruvate Standard Curve (fluorometric). **(c)** 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.

VII. Related Products:

Alanine Aminotransferase (ALT/SGPT) Activity Assay Kit (K752)
EZScreen™ NAD^+/NADH Assay Kit - 384 Well (K958)
EZScreen™ Triglyceride Assay Kit - 384 Well (K952)
EZScreen™ Free Fatty Acid Assay Kit - 384 Well (K956)
EZScreen™ β-Lactamase Activity Assay Kit - 384 Well (K954)
EZScreen™ Lactate Assay Kit - 384 Well (K951)
EZScreen™ Glycogen Assay Kit - 384 Well (K960)
EZScreen™ ATP Assay Kit - 384 Well (K959)

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