Alanine Aminotransferase (ALT or SGPT) Activity Assay Kit (384-Well) (#BN01105)

(Catalog # BN01105; 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 Assay Genie'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). Assay Genie's 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 alanine aminotransferase activity in various biological samples (tissues/cells)
- Measurement of alanine aminotransferase activity in serum and plasma
- Analysis of liver activity/liver injury
- High throughput screening of biological samples

III. Sample Type:

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

IV. Kit Contents:

Components	BN01105	Cap Code	Part Number
ALT Assay Buffer	25 ml	WM	BN01105-1
GenieRed Probe	0.2 ml	Red	BN01105-2
ALT Enzyme Mix	1 vial	Green	BN01105-3
ALT Substrate	1 vial	Orange	BN01105-4
Pyruvate Standard	0.1 ml	Yellow	BN01105-5
ALT Positive Control	1 vial	Blue	BN01105-6

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 small vials prior to opening. Read entire protocol before performing the assay. Keep enzymes and pyruvate standard on ice while in use.

- **ALT Assay Buffer**: Warm to room temperature prior to use. Store at -20°C or 4°C.
- **GenieRed 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.
- **ALT Enzyme Mix**: Reconstitute in 220 µl ddH_2O. 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.
- **ALT Substrate**: 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.
- **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, 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 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.

Note: 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
GenieRed 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}) or fluorescence (Ex/Em = 535/587 nm) in kinetic mode for 60 min or longer at 37°C. 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. For each reaction well (including standard curve and positive control wells), choose two time points (t_1 and t_2) in the linear phase of the reaction progress curves, 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 $\Delta F = RFU_2 - RFU_1$. If sample background control reading is significant then subtract the sample background control reading from each of the sample readings. *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).* 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.

5. Calculation: Plot the Pyruvate Standard curve and 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:

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

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.

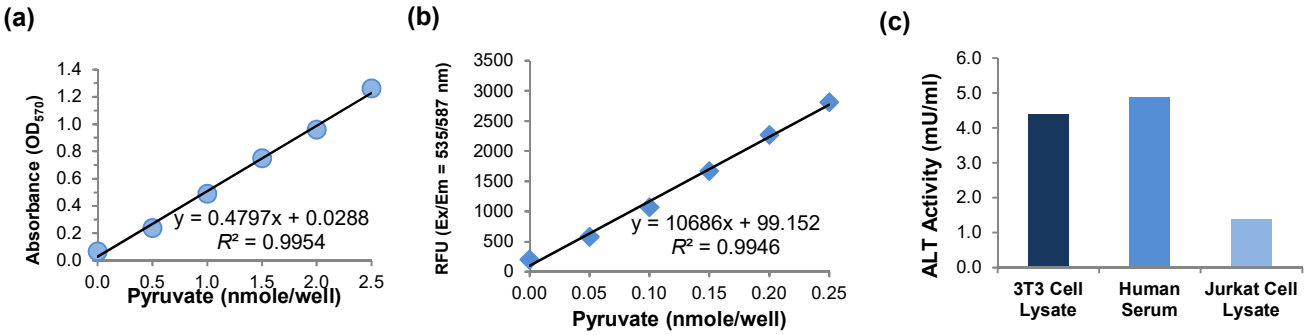

Figure: (a) Pyruvate Standard Curve (colorimetric). **(b)** Pyruvate Standard Curve (fluorometric). **(c)** ALT Activity (mU/ml) in 3T3 Cell Lysate (2.5 µl, 12 mg/ml protein). Pooled Human Serum (2.5 µl), and Jurkat Cell Lysate (1.25 µl, 4 mg/ml protein). Assays were performed according to the kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.