L-Alanine Assay Kit

(Catalog #K652-100; 100 reactions; Store kit at –20°C)

I. Introduction:

Alanine is the 2nd most abundant of the 20 proteinogenic amino acids. Nonessential, being available from dietary sources, it plays a key role in the glucose-alanine cycle between tissues and liver. In muscle and other tissues that degrade amino acids, amino groups are pooled as glutamate by transamination. Glutamate then transfers the amino group to pyruvate via alanine aminotransferase, forming alanine and α-ketoglutarate. The alanine is passed into the blood and transported to the liver. A reverse of the alanine aminotransferase reaction takes place in liver. Pyruvate can be used in gluconeogenesis, to form glucose which may return to other tissues through the circulatory system. There appears to be a correlation between alanine levels and and higher blood pressure, energy intake, cholesterol levels, and body mass index. BioVision's Alanine Assay Kit provides a sensitive detection method of alanine. In the kit, alanine is converted to pyruvate which is specifically detected leading to proportional color (λ=570nm: 0-10 nmol) or fluorescence (Ex/Em 535/587nm: 0-1 nmol) development. Serum concentration: ~24-76 µg/ml (~3-9 nmol/10 µl).

II. Kit Contents:

Components	K652-100	Cap Code	Part No.
Alanine Assay Buffer	25 ml	WM	K652-100-1
Alanine probe	lyophilized	Red	K652-100-2
DMSO	0.4 ml	Brown	K652-100-3
Alanine Converting Enzyme	lyophilized	Purple	K652-100-4
Alanine Development Mix	lyophilized	Green	K652-100-5
Alanine Standard (10 µmol)	lyophilized	Yellow	K652-100-6

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

Alanine Probe: Add 220 µl DMSO. Pipette up and down to dissolve. Protect from light and moisture. Stable for 2 months at -20°C.

Alanine Converting Enzyme, Development Enzyme Mix: Dissolve separately with 220 µl dH_2O. Pipette up and down to dissolve. Aliquot into portions and store at –20°C. Avoid repeated freeze/thaw cycles. Use within two months.

Alanine Standard: Dissolve in 100 µl dH2O to generate 100 mM (100 nmol/µl) Alanine Standard solution. Keep cold while in use. Store at -20°C.

Ensure that the Assay Buffer is at room temperature before use. Keep the Alanine Enzyme Mix on ice during the assay and protect from light.

V. Alanine Assay Protocol:

1. **Alanine Standard Curve:**

Colorimetric: Dilute 10 µl of the 100mM Alanine standard with 990 µl DI H_2O to generate 1 mM standard Alanine. Add 0, 2, 4, 6, 8, 10 µl of the diluted Alanine standard into a 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well standard. Bring the volume to 50 µl with Assay buffer.

Fluorimetric: Dilute standard as for the colorimetric procedure, then take 100 µl of the 1 mM standard and add to 900 µl DI H_2O to make 0.1mM Alanine standard. Add 0, 2, 4, 6, 8, 10 µl of the diluted Alanine standard into a 96-well plate to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well standard. Bring the volume to 50 µl with Assay buffer.

2. **Sample Preparation:**

Tissues or cells (1×10^6) can be homogenized in 100 µl Assay Buffer centrifuge to remove insoluble material at 13,000 g, 10 minutes. 10-50 µl deproteinized serum samples can be directly diluted in the Assay Buffer. Bring sample wells to 50 µl/well with Assay Buffer in a 96-well plate. **For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range**.

3. **Reaction Mix:**

Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

Alanine Measurement	Background Control*
44 µl Assay Buffer	46 µl Assay Buffer
2 µl Alanine Converting Enzyme	----------
2 µl Alanine Development Mix	2 µl Alanine Development Mix
2 µl Alanine Probe**	2 µl Alanine Probe

* Use background control if high levels of pyruvate are suspected to be in the samples.

** For the fluorescent assay dilute the probe 10X to reduce background.

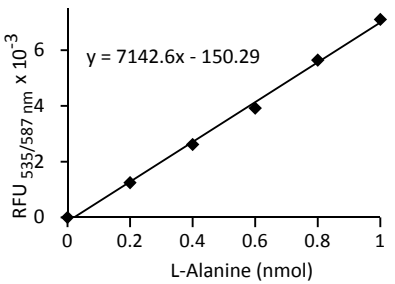
Add 50 µl of the **Reaction Mix** to each well containing the Alanine Standard and test samples. Mix well. Incubate the reaction for 30 min at 37°C, protect from light.

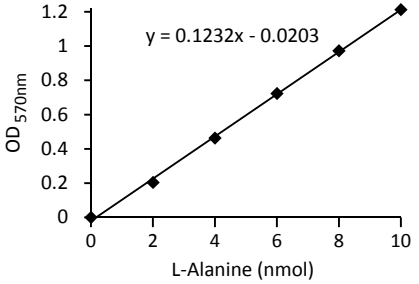
4. Measure O.D. at 570 nm in a microplate reader or fluorescence using Ex/Em 535/587 nm.

5. **Calculation:** Correct background by subtracting the value derived from the 0 Alanine control from all sample readings (The background reading can be significant and must be subtracted from sample readings). Plot Alanine standard Curve, Alanine concentrations of the test samples can then be calculated:

$$\mathbf{C = S_a/S_v \quad nmol/µl, or\ mM}$$

where S_a is the sample amount of unknown (in nmol) from standard curve,
S_v is sample volume (µl) added into the wells.
L-Alanine Molecular Weight is 89.1 g/mol.





VI. Related Products:

NAD/NADH Quantification Kit
ADP/ATP Ratio Assay Kit
Glucose Assay Kit
Ethanol Assay Kit
Pyruvate Assay Kit
Creatine Assay Kit
Ammonia Assay Kit
Triglyceride Assay Kit
Choline/Acetylcholine Quantification Kit
Nitric Oxide Assay Kit
NADP/NADPH Quantification Kit
Ascorbic Acid Quantification Kit
Fatty Acid Assay Kit
Uric Acid Assay Kit
Lactate Assay Kit/ II
L-amino Acid Assay Kit
Free Glycerol Assay Kit
Hemin Assay Kit
Total Antioxidant Capacity (TAC) Assay Kit
Glutathione Detection Kit

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