

# Alanine Colorimetry

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

## I. Introduction:

Alanine is the 2<sup>nd</sup> most abundant of the 20 proteinogenic amino acids. Nonessential, 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 higher blood pressure, energy intake, cholesterol levels, and body mass index. BioVision's Alanine Assay Kit provides a simple, 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 (in DMSO)	0.2 ml	Red	K652-100-2A
Alanine Converting Enzyme	lyophilized	Purple	K652-100-4
Alanine Development Mix	lyophilized	Green	K652-100-5
Alanine Standard (10 μmol)	lyophilized	Orange	K652-100-6

## III. Storage and Handling:

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

## IV. Reagent Reconstitution and General Consideration:

**Alanine Probe:** Ready to use as supplied. Warm to RT to melt frozen DMSO prior to use. Protect from light and moisture. Stable for 2 months at -20°C.

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

**Alanine Standard:** Dissolve in 100 μl dH<sub>2</sub>O to generate 100 mM (100 nmol/μl) Alanine Standard solution. Keep cold while in use. Store at -20°C.

Ensure that the Alanine Assay Buffer is at RT 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 100 μl of the 100mM Alanine Standard with 990 μl DI H<sub>2</sub>O 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 Alanine 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<sub>2</sub>O 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 Alanine Assay buffer.

### 2. Sample Preparation:

Tissues or cells (1x10<sup>6</sup>) 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 reagent for the number of assays to be performed. For each well, prepare a total 50 μl Reaction Mix containing:

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

\* Use Background Control if high levels of pyruvate are suspected to be in the Samples.

\*\* For the fluorescent assay dilute the probe 5-10X to reduce background.

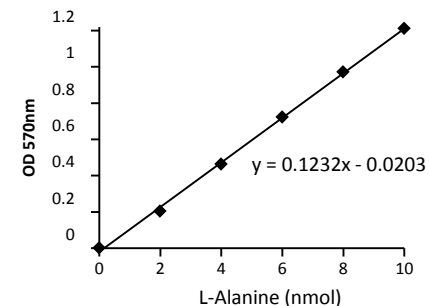
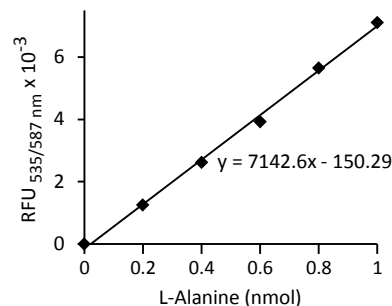
Add 50 μl of the Reaction Mix to each well containing Alanine Standard, Test and Background Control Samples. Mix well. Incubate the reaction for 60 min at 37°C, protect from light.

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:

$$C = S_a/S_v \quad \text{nmol/}\mu\text{l or mM}$$

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

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

**GENERAL TROUBLESHOOTING GUIDE:**

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> <li>• Use of ice-cold assay buffer</li> <li>• Omission of a step in the protocol</li> <li>• Plate read at incorrect wavelength</li> <li>• Use of a different 96-well plate</li> </ul>	<ul style="list-style-type: none"> <li>• Assay buffer must be at room temperature</li> <li>• Refer and follow the data sheet precisely</li> <li>• Check the wavelength in the data sheet and the filter settings of the instrument</li> <li>• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates</li> </ul>
Samples with erratic readings	<ul style="list-style-type: none"> <li>• Use of an incompatible sample type</li> <li>• Samples prepared in a different buffer</li> <li>• Samples were not deproteinized (if indicated in datasheet)</li> <li>• Cell/ tissue samples were not completely homogenized</li> <li>• Samples used after multiple free-thaw cycles</li> <li>• Presence of interfering substance in the sample</li> <li>• Use of old or inappropriately stored samples</li> </ul>	<ul style="list-style-type: none"> <li>• Refer data sheet for details about incompatible samples</li> <li>• Use the assay buffer provided in the kit or refer data sheet for instructions</li> <li>• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated</li> <li>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope</li> <li>• Aliquot and freeze samples if needed to use multiple times</li> <li>• Troubleshoot if needed, deproteinize samples</li> <li>• Use fresh samples or store at correct temperatures till use</li> </ul>
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Allowing the reagents to sit for extended times on ice</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Always check the expiry date and store the components appropriately</li> <li>• Always thaw and prepare fresh reaction mix before use</li> <li>• Refer datasheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> </ul>
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> <li>• Use of partially thawed components</li> <li>• Pipetting errors in the standard</li> <li>• Pipetting errors in the reaction mix</li> <li>• Air bubbles formed in well</li> <li>• Standard stock is at an incorrect concentration</li> <li>• Calculation errors</li> <li>• Substituting reagents from older kits/ lots</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw and resuspend all components before preparing the reaction mix</li> <li>• Avoid pipetting small volumes</li> <li>• Prepare a master reaction mix whenever possible</li> <li>• Pipette gently against the wall of the tubes</li> <li>• Always refer the dilutions in the data sheet</li> <li>• Recheck calculations after referring the data sheet</li> <li>• Use fresh components from the same kit</li> </ul>
Unanticipated results	<ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Samples contain interfering substances</li> <li>• Use of incompatible sample type</li> <li>• Sample readings above/below the linear range</li> </ul>	<ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit</li> <li>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</li> <li>• Concentrate/ Dilute sample so as to be in the linear range</li> </ul>

**Note:** The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.