Oxaloacetate Colorimetric/Fluorometric Assay Kit

(Catalog #K659-100; 100 assays; Store Kit at -20°C)

I. Introduction:

Oxaloacetate (OAA, HOOC-CO-CH_2-COOH) is a TCA cycle intermediate. It precedes citrate which is formed by the transfer of an acetyl group to OAA. OAA is formed by the deamidation of aspartate or condensation of CO_2 with pyruvate or PEP. Since mammals do not possess the enzymatic machinery to form TCA cycle intermediates from acetyl CoA, OAA is one of the anaplerotic entry points via pyruvate and pyruvate carboxykinase. BioVision's Oxaloacetate Assay Kit provides a simple, sensitive and rapid means of quantifying OAA in a variety of samples. In the assay, OAA is converted to pyruvate which is utilized to convert a nearly colorless probe to an intensely colored (λ_{max}= 570nm) and fluorescent (E_x/E_m = 535/587nm) product. The Oxaloacetate Assay Kit can detect 0.1-10nmol (2-200 µM) of OAA.

II. Kit Contents:

Components	K659-100	Cap Code	Part Number
OAA Assay Buffer	25 ml	WM	K659-100-1
OAA Probe (in DMSO)	0.2 ml	Red	K659-100-2A
OAA Enzyme Mix	lyophilized	Purple	K659-100-4
Developer	lyophilized	Green	K659-100-5
OAA Standard (10 µmol)	lyophilized	Yellow	K659-100-6

III. Storage and Handling:

Store kit at –20° C, protect from light. Warm OAA Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Read the entire protocol before the assay.

IV. Reagent Preparation and Storage Conditions:

OAA Probe: Ready to use as supplied. Warm to >18° C to liquefy prior to use. Protect from light and moisture. Store at -20° C. Use within 2 months.

OAA Enzyme Mix, Developer: Add 220 µl OAA Assay Buffer to each component separately. Pipette up and down to dissolve. Aliquot into portions, store at –20° C. Avoid repeated freeze/thaw cycles. Use within 2 months.

OAA Standard: Add 100 µl dH_2O to generate 100 mM (100 nmol/µl) OAA Standard solution. Keep cold. Store at -20° C. OAA slowly decomposes into pyruvate (several hours at room temp, several days at 0°C). This will not affect the assay which measures pyruvate.

V. Assay Protocol:

1. Standard Curve Preparations:

Colorimetric Assay: Dilute OAA Standard to1 nmol/µl by adding 10 µl of the standard to 990µl of dH_2O , mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells on a 96 well plate. Adjust volume to 50 µl/well with OAA Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the standard.

Fluorometric Assay: Dilute OAA standard to 0.1 nmol/µl by adding 10 µl of the standard to 990µl of dH_2O , mix well, then further dilute by adding 10 µl to 90 µl of dH_2O . Add 0, 2, 4, 6, 8, 10 µl into a series of standards well on a 96-well plate. Adjust volume to 50 µl/well to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well OAA Standard.

2. Sample Preparation:

Tissue (20 mg) or cells (2 x 10^6) should be rapidly homogenized with 100µl OAA Assay Buffer. Centrifuge 15000g for 10 min to remove insoluble materials. Enzymes in samples may interfere with the assay. We suggest deproteinizing samples using a perchloric acid/KOH protocol (BioVision, Cat. #K808-200) or 10 kDa molecular weight cut off spin columns (BioVision, Cat # 1997-25). Add 1-50 µl samples into duplicate wells of a 96-well plate and bring volume to 50 µl with Assay Buffer. We suggest testing several doses of your samples to ensure readings are within the standard curve range.

3. Develop:

Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 µl Reaction Mix containing:

	Colorimetric Assay		Fluorometric Assay	
	Sample	**Sample Control**	**Sample**	**Sample Control**
OAA Assay Buffer	44 µl	46 µl	44 µl	46 µl
OAA Enzyme Mix	2 µl	----	2 µl	----
OAA Developer	2 µl	2 µl	2 µl	2 µl
OAA Probe**	2 µl	2 µl	2 µl	2 µl

Notes:

*Pyruvate in samples can cause background color or fluorescence. This background can be subtracted by performing a sample control in the absence of the OAA Enzyme Mix.

**In the fluorometric assay, dilute an aliquot of probe 10x with DMSO to reduce fluorescent background.

Add 50 µl of the Reaction Mix to each well containing the OAA Standard and test samples.

4. Incubate for 30 min at room temperature, protect from light.

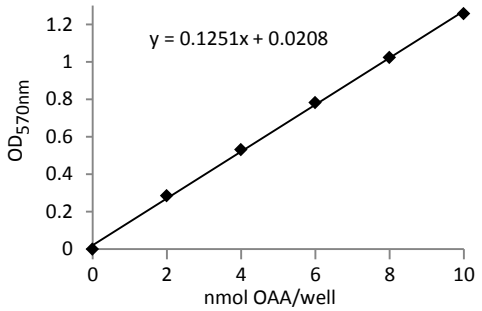
5. Measure OD at 570 nm or fluorescence E_x/E_m at 535/587nm with a 96 well plate reader.

6. Calculation:

Correct background by subtracting the value of the 0 OAA standard from all readings. Next subtract the value of the Sample Control from the samples. (Note: The background reading can be significant and must be subtracted from sample readings). Plot the standard curve. Apply corrected sample readings to the standard curve to get OAA amount in the sample wells. The OAA concentrations in the test samples:

C = Ay/Sv (nmol/µl; or µmol/ml; or mM)

Where: Ay is the amount of OAA (nmol) in your sample from the standard curve.
Sv is the sample volume (µl) added to the sample well.
Oxaloacetic Acid molecular weight: 132.07 g/mol



Oxaloacetate standard curve generated following the kit protocol.

RELATED PRODUCTS:

ADP/ATP Kits	NAD/NADH and NADP/NADPH Assay Kits
CoA/Acetyl CoA Assay Kits	Pyruvate Assay Kit
Malic Acid Assay Kit	Glutamate Assay Kit
α-Ketoglutarate Assay Kit	Lactate Assay Kits
Malic acid Assay Kit	Pyruvate Assay Kit
Glycogen/Starch Assay Kits	Glucose Assay Kit

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

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range

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