Product Information

Alpha-Ketoglutarate Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2216-100 100 assays	Cap Color	Part Number
α-KG Assay Buffer	25 ml	WM	K2216-C-1
α-KG OxiRed Probe (in DMSO)	0.2 ml	Red	K2216-C-2
α-KG Converting Enzyme (lyophilized)	1 vial	Purple	K2216-C-3
α-KG Development Enzyme Mix (lyophilized)	1 vial	Green	K2216-C-4
α-KG Standard (10 µmol, lyophilized)	1 vial	Yellow	K2216-C-5

II. Introduction:

α-Ketoglutarate (α-KG) is a keto acid and is an important intermediate in the Krebs cycle, locating after isocitrate and before succinyl CoA. Anaplerotic reactions can replenish the Krebs cycle by synthesizing α-KG from glutamate through the action of glutamate dehydrogenase or from transamination of glutamate. α-KG is also an important nitrogen transporter. Measurement of α-KG in newborns is an indicator of inborn errors of metabolism.

The Alpha-Ketoglutarate Colorimetric/Fluorometric Assay Kit provides a highly sensitive, fast and convenient way for detection of α-KG levels in various biological samples based on colorimetric and fluorometric method. In the assay, α-KG is transaminated with the production of pyruvate which is utilized to transform a nearly colorless probe to both fluorescence (Ex/Em = 535/587 nm) and color (λmax = 570 nm) product. The kit can detect α-KG in the range of 0.01 to 10 nmoles.

III. Storage and Handling:

Store kit at –20°C, protect from light. Warm α-KG Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening

IV. Reagent Preparation and Storage Conditions:

α-KGProbe: Ready to use as supplied. Warm to room temperature before using to melt frozen DMSO. Protect from light and moisture. Stable for 2 months at -20°C.

α-KGConverting Enzyme, ααα-KGEnzyme Mix: Dissolve with 220 µl α-KG Buffer separately. Pipette up and down to dissolve. Aliquot into vials with sufficient amount for each experiment and store at –20°C. Avoid repeated freeze/thaw cycles. Use within two months.

α-KG Standard: Dissolve in 100 µl dH_2O to generate 100 mM (100nmol/µl) α-KG Standard solution. Keep cold while in use. Store at -20°C.

V. Assay Protocol:

1. Standard Curve Preparations:

Dilute the α-KGStandard to 1 nmol/µl by adding 10µl of the Standard to 990 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of standards wells on a 96-well plate. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Standard.

Fluorometric Assay: Dilute 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 the volume to 50 µl/well to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well.

2. Sample Preparation: Tissue (20 mg) or cells (2 x 10^6) are rapidly homogenized with 100 µl of ice cold α-KG Assay Buffer. Enzymes in samples may interfere with the assay. We suggest deproteinizing samples using a perchloric acid/KOH protocol or 10 kDa molecular weight cut off spin

columns. Add1 - 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 linear range.

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

	Sample	Bkgd. Control
α-KG Assay Buffer	44µl	46µl
α-KG Converting Enzyme	2 µl	---
α-KGEnzyme Mix	2 µl	2 µl
α-KG probe	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the α-KG Standard, samples or background control.

Note:

a Pyruvate generates background. If pyruvate is suspected in your sample, you can do the background control omitting the converting enzyme. The background control can be subtracted from the α-KG reading.

b For fluorometric assay use 0.4 µl α-KG OxiRed Probe and 45.6 µl (47.6 µl for Bkgd Control) α-KG assay buffer to reduce background

4. Incubate for 30 min at 37°C, protect from light.

5. Measure OD at 570 nm or fluorescence using Ex/Em = 535/587 nm.

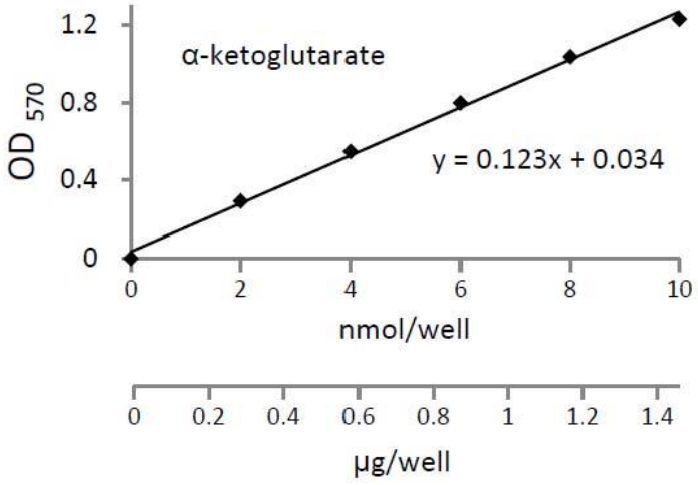
6. Calculation: Correct background by subtracting the value of the 0 α-KGblank from all readings. Plot the standard curve. Apply the corrected sample readings to the standard curve to get α-KGamount in the sample wells.The α-KGconcentrations in the test samples:

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

Where: Ay is the amount of α-KG (nmol) in your sample from the standard curve.

Sv is the sample volume (µl) added to the sample well.

α-Ketoglutarate molecular weight: 146.11.



General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings

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

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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