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Product Information

α-Ketoglutarate Assay Kit

Catalog Number **MAK054**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

α-Ketoglutarate (α-KG) is a key intermediate in the Tricarboxylic Acid (TCA) Cycle, formed by the oxidative decarboxylation of isocitrate by isocitrate dehydrogenase. The synthesis of α-KG from glutamate by glutamate dehydrogenase is a key TCA cycle anaplerotic reaction. α-KG is an important nitrogen transporter. This α-Ketoglutarate Assay Kit provides a simple, sensitive and rapid means for quantifying α-KG in a variety of samples.

α-KG concentration is determined by a coupled enzyme assay, which results in a colorimetric (570 nm)/ fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product, proportional to the α-KG present. Typical detection ranges for this kit are 2–10 nmole (colorimetric) and 0.2–1 nmole (fluorometric).

Components

The kit is sufficient for 100 assays in 96 well plates.

α-KG Assay Buffer Catalog Number MAK054A	25 mL
Flourescent Peroxidase Substrate, in DMSO Catalog Number MAK054B	0.2 mL
α-KG Converting Enzyme Catalog Number MAK054C	1 vl
α-KG Development Enzyme Catalog Number MAK054D	1 vl
α-KG Standard (10 µmole) Catalog Number MAK054E	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader.
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

α-KG Assay Buffer – Allow buffer to come to room temperature before use.

Flourescent Peroxidase Substrate – Warm to room temperature to melt frozen DMSO prior to use. Store protected from light and moisture at –20 °C. Upon thawing, the Flourescent Peroxidase Substrate is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Flourescent Peroxidase Substrate 5 to 10-fold with α-KG Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

α-KG Converting Enzyme – Reconstitute in 220 µL of α-KG Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use and protect from light. Keep cold while in use and protect from light. Use within two months of reconstitution.

α-KG Development Enzyme – Reconstitute in 220 µL of α-KG Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use and protect from light. Use within two months of reconstitution.

α-KG Standard – Reconstitute in 100 µL of water to generate a 100 mM (100 nmole/µL) solution. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

α-KG Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM α-KG standard with 990 µL of water to prepare a 1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM α-KG standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add α-KG Assay Buffer to each well to bring the volume to 50 µL.

α-KG Standards Fluorometric Detection
Prepare a 1 mM standard solution as for the colorimetric assay. Dilute 10 µl of the 1 mM α-KG standard with 90 µl of water to generate a 0.1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µl of the diluted 0.1 mM α-KG standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add α-KG Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Both the colorimetric and fluorometric assays require 50 µL of sample for each reaction (well).

Tissue (20 mg) or cells (2×10^6) can be homogenized in 100 µL ice cold α-KG Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material.Bring samples to a final volume of 50 µL with α-KG Assay Buffer.
Note: Because enzymes in samples may interfere with the assay, samples should be deproteinized with a 10 kDa MWCO spin filter prior to addition to the reaction.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Note: Include a blank sample for each sample by omitting the α-KG Converting Enzyme in the Reaction Mix.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	**Blank Sample**	**Samples and Standards**
α-KG Assay Buffer	46 µL	44 µL
α-KG Converting Enzyme	–	2 µL
α-KG Development Enzyme Mix	2 µL	2 µL
Flourescent Peroxidase Substrate	2 µL	2 µL

1. Add 50 µL of the appropriate Reaction Mix to each of the blank, standard, and test wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at 37 °C. Protect the plate from light during the incubation.

2. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) α-KG standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate α-KG standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of α-KG present in the sample may be determined from the standard curve.

Concentration of α-KG

$$S_a/S_v = C$$

S_a = Amount of α-KG in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of α-KG in sample

α-KG molecular weight: 146.11 g/mole.

Sample Calculation

Amount of α-KG (S_a) = 5.84 nmole

Sample volume (S_v) = 50 μL

Concentration of α-KG in sample

$$5.84 \text{ nmole}/50 \ \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 146.10 \text{ ng/nmole} = 17.06 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range