Technical Bulletin

α-Ketoglutarate Quantitation Kit

Catalogue number MAK541

Product Description

Alpha-ketoglutarate (α-ketoglutarate) is a key molecule in the Krebs cycle determining the overall rate of the citric acid cycle of the organism. As a precursor of glutamate and glutamine, α-ketoglutarate is a central metabolic fuel for cells of the gastrointestinal tract as well. It can decrease protein catabolism and increase protein synthesis to enhance bone tissue formation in the skeletal muscles and can be used in clinical applications.

The α-Ketoglutarate Quantitation Kit offers a sensitive colorimetric assay for quantifying α-ketoglutarate in biological samples. It utilizes an enzyme coupled reaction that releases hydrogen peroxide, which can be detected by the peroxidase substrate at an absorbance of 570 nm.

Components

The kit is sufficient for 200 colorimetric assays in 96-well plates.

- Peroxidase Substrate 1 Vial
  Catalogue Number MAK541A
- Enzyme Mix 1 1 Vial
  Catalogue Number MAK541B
- Enzyme Mix 2 1 Vial
  Catalogue Number MAK541C
- Assay Buffer 10 mL
  Catalogue Number MAK541D
- α-Ketoglutarate Standard (10 mM) 100µL
  Catalogue Number MAK541E
- DMSO 100 µL
  Catalogue Number MAK541F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates and 96-well plate absorbance (590nm) reader for procedure using 96-well plate. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- PBS Buffer (Catalogue Number PPB006 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate to room temperature prior use.

Procedure

All samples and standards should be run in duplicate.

Reagent Preparation

Preparation of Stock Solution

1. Add 50 µL of DMSO into the vial of Peroxidase Substrate to make 200X stock solution.

Preparation of AKG Standard Solution

Note: Fresh reconstitution of the Standard is recommended.

1. α-Ketoglutarate (AKG) standard: Add 10 µL of 10 mM α-Ketoglutarate Standard into 990 µL of PBS to make a 100 µM α ketoglutarate standard solution (AKG1).
2. Perform 1:2 serial dilutions to get the diluted α-ketoglutarate standards (AKG2 – AKG7) as shown in Table 1.

Table 1.
Serial dilution of AKG Standard Solutions

Dilution	AKG Standard Volume (µL)	Serial Dilution Source	PBS Volume(µL)	Conc. (µM)
AKG1	300	-	0	100
AKG2	150	From AKG1	150	50
AKG3	150	From AKG2	150	25
AKG4	150	From AKG3	150	12.5
AKG5	150	From AKG4	150	6.25
AKG6	150	From AKG5	150	3.12
AKG7	150	From AKG6	150	1.56

Preparation of Working Solution

Note: Prepare immediately before use in assay reaction. The working solution is enough for one 96-well plate.

1. Add 5 mL of Assay Buffer into one Enzyme Mix 1 vial then mix well.
2. Add 100 µL of purified water into one Enzyme Mix 2 vial and mix well.
3. Transfer entire vial (100 µL) of Enzyme Mix 2 and 25 µL of the 200x Peroxidase Substrate stock solution into the vial of Enzyme Mix 1 and mix well.

Assay Reaction

1. Add 50 µL of samples, standards, and blanks to separate wells.
2. Add 50 µL of the Working Solution to each well containing a sample, standard, and blank.
3. Incubate the reaction mixture at 37 °C for 60 - 90 minutes.

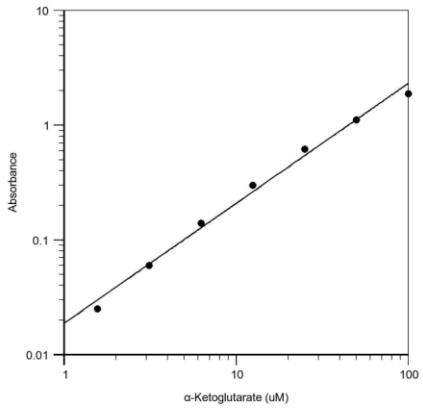
Measurement

1. Monitor the absorbance increase with an absorbance plate reader with path check on at OD of 570 nm.

Results

1. The reading (Absorbance) obtained from the blank standard well is used as a negative control.
2. Subtract this value from the other standards readings to obtain the base-line corrected values.
3. Plot the standards readings to obtain the standard curve.
4. Use linear regression to calculate the α-Ketoglutarate concentration.

Figure 1.
Typical α-ketoglutarate Standard Curve



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