Amplite™ Colorimetric α-Ketoglutarate Quantitation Kit

Catalog number: 10085
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B1: Enzyme Mix 1	Freeze (<-15 °C), Minimize light exposure	2 bottles (lyophilized powder)
Component B2: Enzyme Mix 2	Freeze (<-15 °C), Minimize light exposure	2 vials (lyophilized powder)
Component C: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)
Component D: α-Ketoglutarate Standard	Freeze (<-15 °C), Minimize light exposure	10 mM (100 µL)
Component E: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

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. Alpha-ketoglutarate is used for kidney disease; intestinal and stomach disorders, including bacterial infections; liver problems; cataracts; and recurring yeast infections. It is also used for improving the way kidney patients receiving hemodialysis treatments process protein. AAT Bioquest's Amplite™ Colorimetric 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 Amplite™ Red in an absorbance microplate reader at 570 nm.

AT A GLANCE

Protocol summary

1. Prepare test samples along with serially diluted α-ketoglutarate standards (50 µL)
2. Add equal volume of α-Ketoglutarate working solution (50 µL)
3. Incubate at 37°C for 60 - 90 minutes
4. Monitor absorbance intensity at 570 nm

Important Thaw one vial of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	575 nm
Recommended plate:	Clear bottom
Instrument specification(s):	Path check

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Amplite™ Red stock solution (200X):
Add 50 µL of DMSO (Component E) into the vial of Amplite™ Red (Component A) to make 200X stock solution.

PREPARATION OF STANDARD SOLUTION

α-Ketoglutarate standard

For convenience, use the Serial Dilution Planner:

https://www.aatbio.com/tools/serial-dilution/10085

Add 10 µL of 10 mM α-Ketoglutarate Standard (Component D) into 990 µL of PBS to get 100 µM α-ketoglutarate standard solution (AKG7). Then perform 1:2 serial dilutions to get serially diluted α-ketoglutarate standards (AKG6 - AKG1).

PREPARATION OF WORKING SOLUTION

1. Add 5 mL Assay Buffer (Component C) into one Enzyme Mix 1 bottle (Component B1) and mix well.

2. Add 100 µL of ddH_2O into one Enzyme Mix 2 vial (Component B2) and mix well.

3. Transfer entire vial (100 µL) of Enzyme Mix 2 and 25 µL of 200X Amplite™ Red stock solution into the vial of Enzyme Mix 1 and mix well to make α-Ketoglutarate working solution.

Note The 5 mL α-Ketoglutarate working solution is enough for one 96-well plate. It is not stable, use it promptly.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of α-ketoglutarate standards and test samples in a 96-well clear bottom microplate. AKG= α-Ketoglutarate Standard (AKG1 - AKG7, 1.563 to 100 µM), BL=Blank Control, TS=Test Sample.

BL	BL	TS	TS
AKG1	AKG1	...	...
AKG2	AKG2	...	...
AKG3	AKG3		
AKG4	AKG4		
AKG5	AKG5		
AKG6	AKG6		
AKG7	AKG7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
AKG1 - AKG7	50 µL	Serial Dilutions (1.563 to 100 µM)
BL	50 µL	Assay Buffer (Component C)
TS	50 µL	test sample

1. Prepare α-ketoglutarate standards (AKG), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

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2. Add 50 µL of α-Ketoglutarate working solution to each well of α-ketoglutarate standard, blank control, and test samples to make the total α-ketoglutarate assay volume of 100 µL/well. For a 384-well plate, add 25 µL of α-Ketoglutarate working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction mixture at 37°C for 60 - 90 minutes.

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

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate α-Ketoglutarate samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

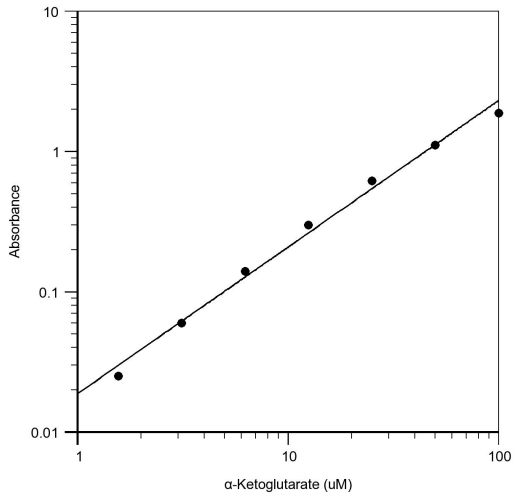

Figure 1. Alpha-ketoglutarate dose response was measured with Amplite™ Colorimetric α-Ketoglutarate Quantitation Kit in a 96-well clear bottom plate using a SpectraMax microplate reader (Molecular Devices) with path check on mode.

DISCLAIMER