Amplite™ Fluorimetric α-Ketoglutarate Quantitation Kit

Catalog number: 10087
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	1 vial (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. Amplite™ Fluorimetric α-Ketoglutarate Quantitation Kit offers a sensitive fluorimetric assay for quantifying α-ketoglutarate in biological samples. It utilizes an enzyme coupled reaction that releases hydrogen peroxide, which can be detected with Amplite™ Red at Ex/Em ~540/590 nm (red fluorescence).

AT A GLANCE

Protocol Summary

1. Prepare α-Ketoglutarate standards or test samples (50 µL)
2. Add α-Ketoglutarate Assay working solution (50 µL)
3. Incubate at 37 °C for 30 to 60 minutes
4. Read fluorescence intensity at Ex/Em = 540/590 nm

Important Thaw all the kit components to room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	540 nm
Emission	590 nm
Cutoff	570 nm
Recommended plate	Solid black

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.

Amplite™ Red stock solution (200X)
Add 50 µL of DMSO (Component E) into one vial of Amplite™ Red (Component A) and mix them well.

Note Store unused Amplite™ Red stock solution (200X) at -20°C, avoid light and repeated freeze-thaw cycles.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/10087

alpha-Ketoglutarate standard
Add 2.5 uL of alpha-Ketoglutarate standard in 225 uL of PBS buffer to make 100 uM. Perform 1:3 serial dilutions to get approximately 33, 11, 3.7, 1.23, 0.41 and 0.1 µM serially diluted α-ketoglutarate standards.

PREPARATION OF WORKING SOLUTION

α-Ketoglutarate Assay working solution
Add 5 mL Assay Buffer (Component C) into one Enzyme Mix 1 bottle (Component B1) and mix well. Add 100 µL of ddH_2O into one Enzyme Mix 2 vial (Component B2) and mix well. Transfer entire vial (100 µL) of Enzyme Mix 2 and 25 µL of 200X Amplite™ Red stock solution (200X) into the vial of Enzyme Mix 1 and mix well.

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

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of α-Ketoglutarate standards and test samples in a solid black 96-well microplate. AKG= α-Ketoglutarate Standards (AKG1 - AKG7, 0.1 to 100 µM), BL=Blank Control, TS=Test Samples.

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 (0.1 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.
2. Add 50 µL of α-Ketoglutarate Assay working solution to each well of α-Ketoglutarate standard, blank control, and test samples to make the total 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 at 37 ºC for 30 - 60 minutes.
4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540/590 nm (Cutoff = 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

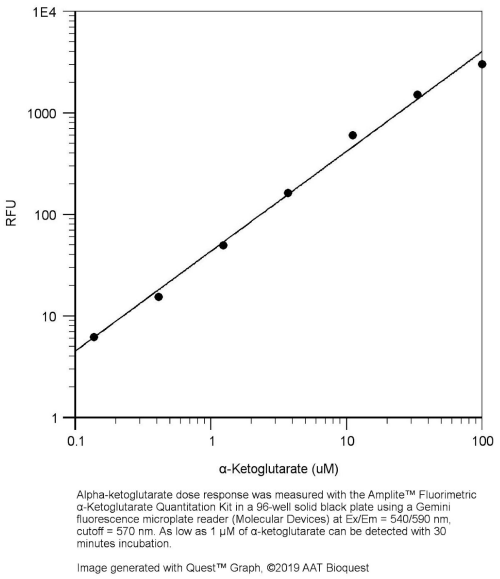

Figure 1. Alpha-ketoglutarate dose response was measured with the Amplite™ Fluorimetric α-Ketoglutarate Quantitation Kit in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices) at Ex/Em = 540/590 nm, cutoff = 570 nm. As low as 1 µM of α-ketoglutarate can be detected with 30 minutes incubation.

DISCLAIMER

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