# Amplite™ Colorimetric α-Ketoglutarate Quantitation Kit

**Red Color**

| *Ordering Information* | *Storage Conditions* | *Instrument Platform* |
|---|---|---|
| Cat#:10085 (200 Assays) | Keep in freezer and protect from light | Absorbance microplate readers |

## Introduction

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.

## Kit Components

| **Components** | **Amount** |
|---|---|
| Component A: Amplite™ Red | 1 vial |
| Component B1: Enzyme Mix 1 | 2 bottles (lyophilized powder) |
| Component B2: Enzyme Mix 2 | 2 vials (lyophilized powder) |
| Component C: Assay Buffer | 1 bottle (10 mL) |
| Component D: α-Ketoglutarate Standard | 10 mM (100 µL) |
| Component E: DMSO | 1 vial (100 µL) |

## Assay Protocol for One 96-Well Plate

### Brief Summary

**Prepare test samples (50 µL) along with serially diluted α-ketoglutarate standards (50 µL) → Add equal volume of Assay Mixture (50 µL) → Incubate at 37 ºC for 60-90 minutes → Monitor absorbance intensity at 570 nm**

*Note: Thaw one vial of each kit component at room temperature before starting the experiment.*

**1. Prepare α-ketoglutarate assay mixture:**

1.1 Make Amplite™ Red stock solution (200X): Add 50 µL of DMSO (**Component E**) into Amplite™ Red (**Component A**) to make 200X stock solution.

1.2 Make assay mixture:

1.2.1 Add 5 mL Assay Buffer (**Component C**) into one Enzyme Mix1 bottle (**Component B1**) and mix well.

1.2.2 Add100 µL of $ddH_2O$ into one Enzyme Mix2 vial (**Component B2**) and mix well.

1.2.3 Transfer entire vial (100 µL) of Enzyme Mix2 (from Step 1.2.2), and 25 µL of 200X Amplite™ Red stock solution (from Step 1.1) into the vial of Enzyme Mix 1(from Step 1.2.1) and mix well.

*Note 1: The 5 mL assay mixture is enough for one 96-well plate. It is not stable, use it promptly.*

*Note 2: Store unused 200X Amplite™ Red stock solution at -20ºC, avoid light and repeated freeze-thaw cycles.*

**2. Prepare serially diluted α-ketoglutarate standards and test samples:**

2.1 Prepare α-ketoglutarate standards: Add 10 µL of 10 mM α-Ketoglutarate Standard (**Component D**) into 990 µL of PBS to get 100 µM α-ketoglutarate solution. Perform 1:2 serial dilutions to get 50, 25, 12.5, 6.25, 3.125 and 1.563 µM serially diluted α-ketoglutarate standards.

2.2 Add α-ketoglutarate containing samples and serially diluted α-ketoglutarate standards into a 96-well clear bottom microplate according to Tables 1 and 2.


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**Table 1.** Layout of α-ketoglutarate standards and test samples in a 96-well clear bottom microplate.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BL | BL | TS | TS | …. | …. | | | | | | |
| AKG 1 | AKG 1 | …. | …. | …. | …. | | | | | | |
| AKG 2 | AKG 2 | | | | | | | | | | |
| AKG 3 | AKG 3 | | | | | | | | | | |
| AKG 4 | AKG 4 | | | | | | | | | | |
| AKG 5 | AKG 5 | | | | | | | | | | |
| AKG 6 | AKG 6 | | | | | | | | | | |
| AKG 7 | AKG 7 | | | | | | | | | | |

*Note: AKG= α-Ketoglutarate Standard, BL=Blank Control (PBS), TS=Test Sample.*

**Table 2.** Reagent composition for each well

| **α-Ketoglutarate Standard** | **Blank Control** | **Test Sample** |
|---|---|---|
| Serial Dilutions: 50 µL | Assay Buffer: 50 µL | 50 µL |

*Note: Add the serial dilutions of α-ketoglutarate standards from 1.56 µM to 100 µM into wells from AKG 1 to AKG 7.*

**3. Run α-ketoglutarate assay:**

3.1 Add 50 µL of assay mixture (from Step 1.2.3) into each well of α-ketoglutarate standard, blank control and test samples (see Step 2.2) to make the total α-ketoglutarate assay volume of 100 µL/well.
*Note: For a 384-well plate, add 25 µL of sample, 25 µL of assay mixture (from Step 1.2.3) into each well.*

3.2 Incubate the reaction mixture at 37 ºC for 60 – 90 minutes.

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

## Data Analysis

The absorbance reading in blank wells (with assay buffer only) is used as a control, and is subtracted from the values of the wells with the α-ketoglutarate standards and test samples. The α-ketoglutarate standard curve is shown in Figure 1. Calculate the concentrations of the samples according to the α-ketoglutarate standard curve.
*Note: The absorbance background increases with time, thus it is important to subtract the absorbance intensity value of the blank wells for each data point.*

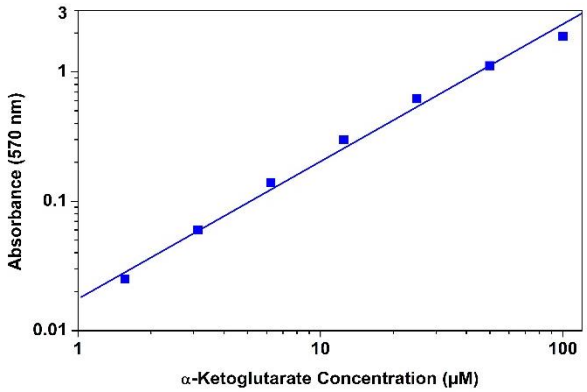


**Figure 1.** Alpha-ketoglutarate dose response was measured with the Amplite™ Colorimetric α-Ketoglutarate Quantitation Kit in a 96-well clear bottom plate using a SpectraMax microplate reader (Molecular Devices) with path check on mode. As low as 3 µM α-ketoglutarate can be detected with 60 minutes incubation.

## References

1. N. Wu, M. Yang, U. Gaur, H. Xu, Y. Yao, and D. Li. Alpha-Ketoglutarate: Physiological Functions and Applications. Biomol Ther (Seoul). 2016 Jan; 24(1): 1–8.
2. Cunningham GA, McClenaghan NH, Flatt PR, Newsholme P. "L-alanine induces changes in metabolic and signal transduction gene expression in a clonal rat pancreatic beta-cell line and protects from pro-inflammatory cytokine-induced apoptosis." *Clin Sci (Lond)*. 2005 Nov;109 (5):447-55.
3. Sann L, Ruitton A, Mathieu M, Bourgeois J, Genoud J. "Effect of intravenous L-alanine administration on plasma glucose, insulin and glucagon, blood pyruvate, lactate and beta-hydroxybutyrate concentrations in newborn infants. Study in term and preterm newborn infants." Acta Paediatr Scand. 1978 May;67(3):297-302.