**Amplite™ Fluorimetric α-Ketoglutarate Quantitation Kit**

**Component** | **Storage** | **Amount**
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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), Minimize light exposure | 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 α-Ketoglutarate standards or test samples (50 µL)
2. Add α-Ketoglutarate Assay working solution (50 µL)
3. Incubate at 37 ºC 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**

- **Instrument:** 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**

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

Perform 1:3 serial dilutions to get approximately 30, 10, 3, 1, 0.3 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 ddH₂O 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 30 µM), BL=Blank Control, TS=Test Samples.

<table>
<thead>
<tr>
<th>Well</th>
<th>Volume</th>
<th>Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL</td>
<td>50 µL</td>
<td>Serial Dilutions (0.1 to 30 µM)</td>
</tr>
<tr>
<td>AKG1</td>
<td>50 µL</td>
<td>Assay Buffer (Component C)</td>
</tr>
<tr>
<td>AKG2</td>
<td>50 µL</td>
<td>test sample</td>
</tr>
</tbody>
</table>

Table 2. Reagent composition for each well.

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**

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards’ readings to obtain the baseline 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:


![Image of graph](http://example.com/graph.png)

**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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