

# Amplite™ Fluorimetric $\alpha$ -Ketoglutarate Quantitation Kit

## \*Red Fluorescence\*

Ordering Information	Storage Conditions	Instrument Platform
Cat#:10087 (200 Assays)	Keep in freezer and protect from light	Fluorescence microplate readers

### Introduction

Alpha-ketoglutarate ( $\alpha$ -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,  $\alpha$ -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™ Fluorimetric  $\alpha$ -Ketoglutarate Quantitation Kit offers a sensitive fluorimetric assay for quantifying  $\alpha$ -ketoglutarate in biological samples. It utilizes an enzyme coupled reaction that releases hydrogen peroxide, which can be detected by Amplite™ Red to generate strong red fluorescence.

### 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: $\alpha$ -Ketoglutarate Standard	10 mM (100 $\mu$ L)
Component E: DMSO	1 vial (100 $\mu$ L)

### Assay Protocol for One 96-Well Plate

#### Brief Summary

**Prepare test samples (50  $\mu$ L) along with serially diluted  $\alpha$ -ketoglutarate standards (50  $\mu$ L)**  
**→ Add equal volume of Assay Mixture (50  $\mu$ L) → Incubate at 37 °C for 30-60 minutes**  
**→ Read fluorescence intensity at Ex/Em = 540/590 nm**

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

#### 1. Prepare $\alpha$ -ketoglutarate assay mixture:

- 1.1 **Make Amplite™ Red stock solution (200X):** Add 50  $\mu$ L 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 Add 100  $\mu$ L of ddH<sub>2</sub>O into one Enzyme Mix2 vial (**Component B2**) and mix well.
  - 1.2.3 Transfer entire vial (100  $\mu$ L) of Enzyme Mix2 (from Step 1.2.2), and 25  $\mu$ L 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 -20°C, avoid light and repeated freeze-thaw cycles.*

#### 2. Prepare serially diluted $\alpha$ -ketoglutarate standards and test samples:

- 2.1 **Prepare  $\alpha$ -ketoglutarate standards:** Add 10  $\mu$ L of 10 mM  $\alpha$ -Ketoglutarate Standard (**Component D**) into 990  $\mu$ L of PBS to get 100  $\mu$ M  $\alpha$ -ketoglutarate solution. Perform 1:3 serial dilutions to get approximately 30, 10, 3, 1, 0.3 and 0.1  $\mu$ M serially diluted  $\alpha$ -ketoglutarate standards.
- 2.2 Add  $\alpha$ -ketoglutarate containing samples and serially diluted  $\alpha$ -ketoglutarate standards into a 96-well solid black microplate according to Tables 1 and 2.

