EnzyChrom™ α-Ketoglutarate Assay Kit (EAKG-100)

Quantitative Colorimetric/Fluorimetric α-Ketoglutarate Determination

DESCRIPTION

α-Ketoglutarate (αKG) is a crucial intermediate in the citric acid (TCA) cycle and plays an important role in amino acid formation, nitrogen transport, and oxidation reactions. αKG can modulate muscle and bone growth as well as the aging process, and has also been investigated for its potential to enhance anticancer immune functions. BioAssay Systems' α-ketoglutarate assay uses a single Working Reagent that combines αKG deamination, pyruvate oxidation and hydrogen peroxide determination. The change in OD570nm or fluorescence intensity at $\lambda_{ex/em}$ = 530/585nm is directly proportional to the αKG present in the sample.

KEY FEATURES

Sensitive and accurate. Linear detection range in 96-well plate: 1.3 to 200 µM for colorimetric assays and 0.08 to 20 µM for fluorimetric assays.

Fast and convenient. Room temperature assay with a 30 min incubation. No 37°C incubator is needed.

High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of αKG in serum, tissue and cell lysates.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Developer:	5 mL	**Enzyme Mix:**	100 µL
Dye Reagent:	120 µL	**Cosubstrate:**	100 µL
Standard:	100 µL (5mM)		

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation

Serum: Centrifuge through 10 kDa molecular weight cut-off filter (e.g. Amicon Ultra-0.5, Ultracel-10 Membrane, 10 kDa from Millipore). Tissue and cell lysates can be assayed directly.

For unknown samples, it is prudent to test several dilutions to determine an optimal sample dilution factor *n*.

All samples can be stored at -20 to -80°C for at least one month.

Reagent Preparation

Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay.

COLORIMETRIC ASSAY

1. Sample Preparation. This internal standard method needs three separate reactions: 1) Sample plus Standard ("IS"), 2) Sample alone ("S") and 3) Sample Blank ("SB").

   For the "IS" wells, add 1 µL of 5 mM Standard to 50 µL of sample, mix well, and transfer 50 µL to the well. For "S" and "SB" wells, add 2 µL dH_2O. to 100 µL of sample, mix well, and transfer 50 µL to the "S" and "SB" wells, respectively.

2. Prepare Working Reagent (WR). For "IS" and "S" wells, prepare sufficient WR by mixing, for each well, 50 µL Developer, 1 µL Enzyme Mix, 1 µL Cosubstrate, and 1 µL Dye Reagent.

   Prepare sufficient Blank Working Reagent (BWR) for the "SB" wells by mixing, for each well, 50 µL Developer, 1 µL dH_2O (no Enzyme), 1 µL Cosubstrate, and 1 µL Dye Reagent.

   Add 50 µL WR and 50 µL BWR to the appropriate wells and tap the plate briefly to mix.

3. Read Optical Density at 570 nm immediately (0 min) and at 30 min, or record kinetics for 30 min.

FLUORIMETRIC ASSAY

1. Sample Preparation: Prepare 200 µM Standard as needed by mixing the 5 mM Standard and dH_2O. For the "IS" wells, add 1.5 µL of 200 µM Standard to 50 µL of sample, mix well, and transfer 50 µL to the well. For "S" and "SB" wells, add 3 µL dH_2O to 100 µL of sample, mix well, and transfer 50 µL to the "S" and "SB" wells, respectively.

2. Prepare Working Reagent (WR). For "IS" and "S" wells, prepare sufficient WR by mixing, for each well, 50 µL Developer, 1 µL Enzyme Mix, 1 µL Cosubstrate, and 0.25 µL Dye Reagent. Prepare sufficient Blank Working Reagent (BWR) for the "SB" wells by mixing, for each well, 50 µL Developer, 1 µL dH_2O, 1 µL Cosubstrate, and 0.25 µL Dye Reagent (no Enzyme).

   Add 50 µL WR and 50 µL BWR to the appropriate wells and tap the plate briefly to mix.

3. Read fluorescence ($\lambda_{ex/em}$ = 530/585 nm) immediately (0 min) and at 30 min, or record kinetics for 30 min.

CALCULATION

For Colorimetric Assay:

$$[\alpha KG] = \frac{\Delta OD_S - \Delta OD_{SB}}{\Delta OD_{IS} - \Delta OD_S} \times n \times 98.04 \quad (\mu M)$$

ΔOD_S, ΔOD_{SB}, and ΔOD_{IS} are the ΔOD (30min - 0min) values of the Sample, Sample Blank and Sample plus Internal Standard, respectively. 98.04 is the spiked internal standard concentration. *n* is the sample dilution factor.

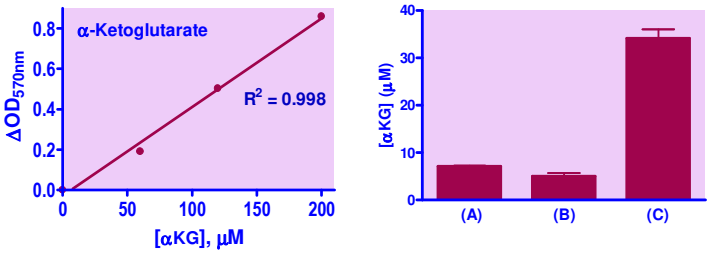
For Fluorimetric Assay:

$$[\alpha KG] = \frac{\Delta F_S - \Delta F_{SB}}{\Delta F_{IS} - \Delta F_S} \times n \times 5.83 \quad (\mu M)$$

ΔF_S, ΔF_{SB}, and ΔF_{IS} are the ΔF (30min - 0min) values of the Sample, Sample Blank and Sample plus Internal Standard, respectively. 5.83 is the spiked internal standard concentration. *n* is the sample dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, clear flat-bottom 96-well plates, black flat-bottom uncoated 96-well plates, centrifuge tubes, and plate reader. For serum samples, 10 kDa molecular weight cut-off filters.



Left: Colorimetric Assay αKG Standard Curve. ***Right***: Cell lysate and tissue lysate were assayed directly. Serum prepared according to the protocol. Samples were assayed in duplicate using fluorimetric method. Results: (A) HepG2 (liver) cell lysate: 7.08 ± 0.22 µM; (B) Mouse brain tissue lysate: 5.03 ± 0.93 µM; (C) Human serum: 34.1 ± 2.8 µM

LITERATURE

1. Chin RM et al (2014). The metabolite α-ketoglutarate extends lifespan by inhibiting ATP synthase and TOR. Nature. 510(7505):397–401.

2. Liu N et al (2023). Supplementation with α-ketoglutarate improved the efficacy of anti-PD1 melanoma treatment through epigenetic modulation of PD-L1. Cell Death and Disease. 14(2).

3. Rzeski W et al (2012). Alpha-ketoglutarate (AKG) inhibits proliferation of colon adenocarcinoma cells in normoxic conditions. Scandinavian Journal of Gastroenterology. 47(5):565–571.