

α-Ketoglutarate Assay Kit

(Catalog #K677-100; 100 assays; Store Kit at -20°C)

I. Introduction:

α-Ketoglutarate (α-KG) is a key intermediate in the Krebs cycle, coming after isocitrate and before succinyl CoA. Anaplerotic reactions replenish the cycle by synthesizing α-KG from transamination of glutamate, or through the action of glutamate dehydrogenase. α-KG is an important nitrogen transporter. Being a key intermediate, it is one of the organic acids measured in newborns as an indicator of inborn errors of metabolism. BioVision's α-Ketoglutarate Assay Kit provides a simple, sensitive and rapid means for quantifying α-KG in a variety of samples. In the assay, α-ketoglutarate is transaminated with the generation of pyruvate which is utilized to convert a nearly colorless probe to both color ($\lambda_{max} = 570 \text{ nm}$) and fluorescence (Ex/Em = 535/587 nm). The α-Ketoglutarate Assay Kit is useful for detecting α-ketoglutarate in the range of 0.01 to 10 nmoles.

II. Kit Contents:

Components	K677-100	Cap Code	Part Number
α-KG Assay Buffer	25 ml	WM	K677-100-1
α-KG OxiRed Probe	lyophilized	Red	K677-100-2
DMSO (Anhydrous)	0.4 ml	Brown	K677-100-3
α-KG Converting Enzyme	lyophilized	Purple	K677-100-4
α-KG Development Enzyme Mix	lyophilized	Green	K677-100-5
α-KG Standard (10 μmol)	lyophilized	Yellow	K677-100-6

III. Storage and Handling:

Store kit at -20°C, protect from light. Warm α-KG Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

IV. Reagent Preparation and Storage Conditions:

α-KG Probe: Add 220 μl DMSO. Pipette up and down to dissolve. Protect from light and moisture. Stable for 2 months at -20°C.

α-KG Converting Enzyme, α-KG Enzyme Mix: Dissolve with 220 μl α-KG Buffer separately. Pipette up and down to dissolve. Aliquot into vials with sufficient amount for each experiment and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months.

α-KG Standard: Dissolve in 100 μl dH2O to generate 100 mM (100 nmol/μl) α-KG Standard solution. Keep cold while in use. Store at -20°C.

V. Assay Protocol:

1. Standard Curve Preparations:

Dilute the α-KG Standard to 1 nmol/μl by adding 10 μl of the Standard to 990 μl of dH2O, mix well. Add 0, 2, 4, 6, 8, 10 μl into a series of standards wells on a 96 well plate. Adjust volume to 50 μl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Standard.

2. Sample Preparation:

Tissue (20 mg) or cells (2×10^6) are rapidly homogenized with 100 μl of ice cold PBS or other buffer (pH 6.5-8). Enzymes in samples may interfere with the assay. We suggest deproteinizing samples using a perchloric acid/KOH protocol (BioVision, Cat.# K808-200) or 10 kD molecular weight cut off spin columns (BioVision, Cat # 1997-25). Add 1-50 μl samples into duplicate wells of a 96-well plate and bring volume to 50 μl with Assay Buffer. We suggest testing several doses of your samples to ensure readings are within the linear range.

3. Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 μl Reaction Mix containing:

	Sample	Bkgd. Control*
α-KG Assay Buffer	44 μl	46 μl
α-KG Converting Enzyme	2 μl	-----
α-KG Enzyme Mix	2 μl	2 μl
α-KG probe	2 μl	2 μl

Add 50 μl of the Reaction Mix to each well containing the α-KG Standard, samples or background control*.

***Note:** Pyruvate generates background. If pyruvate is suspected in your sample, you can do the background control omitting the converting enzyme. The background control can be subtracted from the α-KG reading.

4. Incubate for 30 minutes at 37°C, protect from light.

5. Measure OD at 570 nm or fluorescence using Ex/Em=535/587 nm.

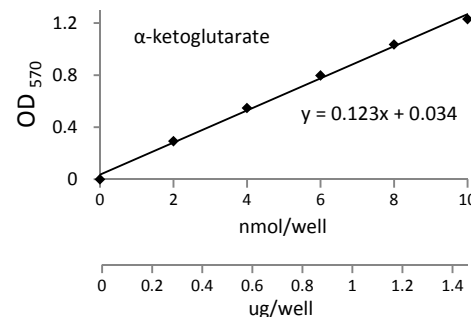
6. **Calculation:** Correct background by subtracting the value of the 0 α-KG blank from all readings. Plot the standard curve. Apply the corrected sample readings to the standard curve to get α-KG amount in the sample wells. The α-KG concentrations in the test samples:

$$C = Ay/Sv \text{ (nmol/}\mu\text{l; or } \mu\text{mol/ml; or mM)}$$

Where: Ay is the amount of α-KG (nmol) in your sample from the standard curve.

Sv is the sample volume (μl) added to the sample well.

α-Ketoglutarate molecular weight: 146.11



α-Ketoglutarate standard curve generated using this kit protocol.

VI. RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents	Cell Proliferation & Senescence Kits
Glucose, Sucrose Assay Kit	Cholesterol, LDL/HDL Assay Kits
Glutathione Assay Kit	Ethanol and Uric Acid Assay Kit
NAD/NADH and NADP/NADPH Assay Kit	Lactate Assay Kits
TAC Total Antioxidant Capacity	Mono or Polysaccharide Assay Kits
Malic acid Assay Kit	Pyruvate Assay Kit
Glycogen Assay Kit	Fatty Acid Assay Kit
Galactose Assay Kit	Nitric Oxide Assay Kit
Fructose Assay Kit	Iron Assay Kit