

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 (570 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 probe	lyophilized	Red	K677-100-2
DMSO (Anhydrous)	0.4 ml	Brown	K677-100-3
α-KG Enzyme Mix	lyophilized	Green	K677-100-4
α-KG Standard (10 μmol)	lyophilized	Yellow	K677-100-5

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 Enzyme Mix: Dissolve with 220 μl dH₂O. 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 samples: (10-100 mg) should be rapidly homogenized with an equivalent volume 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 standard curve range.

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

	Positive Sample	Control
α-KG Assay Buffer	46 μl	48 μl
α-KG Enzyme Mix	2 μl	---
α-KG probe	2 μl	2 μl

Add 50 μl of the Reaction Mix to each well containing the α-KG Standard and test samples.

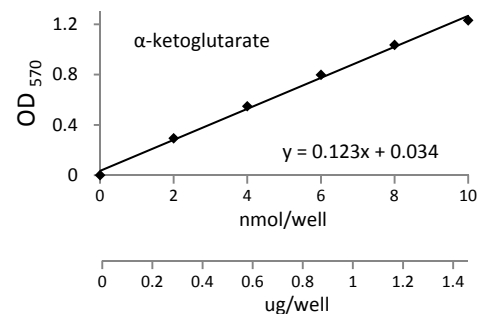
- Incubate for 30 minutes at 37°C, protect from light.
- Measure OD at 570 nm or fluorescence using Ex/Em=535/587 nm.
- Calculation:** Correct background by subtracting the value of the 0 α-KG blank from all sample readings. Next subtract the value of the No Enzyme Control from the Positive sample. (Note: The background reading can be significant and must be subtracted from sample readings). Plot the standard curve. Then 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:

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

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:

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| Apoptosis Detection Kits & Reagents | Cell Proliferation & Senescence Kits |
| Glucose and 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 |
| Glucose Assay Kit | Glycogen Assay Kit |
| Sucrose assay Kit | Fatty Acid Assay Kit |
| Galactose Assay Kit | Nitric Oxide Assay Kit |
| Fructose Assay Kit | Iron Assay Kit |
| Maltose Assay Kit | Urea Assay Kit |