BIOMEDICAL RESEARCH SERVICE CENTER
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α-Ketoglutarate (α-KG) Assay Kit (Cat #: A-132)

COMPONENTS: α-KG Assay Solution- 10 ml (for 100 wells), store at -70ºC
Ammonium (500x)- 0.1 ml, store at -70ºC
GLDH (50x; glutamate dehydrogenase)- 0.2 ml, store at -70ºC
NADPH (100x)- 0.1 ml (brown vial), store at -70ºC **(avoid light exposure)**
20 mM α-KG- 0.2 ml, store at -20ºC or -70ºC
PEG Solution- 5 ml; store at 4°C **(viscous; pipette solution with a cut tip)**

PRODUCT DESCRIPTION: The α-KG assay is based on the difference in the UV absorption spectra between the oxidized and reduced forms of NADP+/NADPH at 340 nm (assay range 10 – 200 μM α-KG). The assay requires the use of UV-transparent 96-well plate or 0.1 ml quartz cuvette for measuring changes in O.D.340 nm. Reagents are stable for at least 1 year if stored and handled properly.

PROTOCOLS

Preparation of serum/plasma and cell culture medium: These samples are deproteinized by PEG precipitation. Mix 50 μl of each sample with 50 μl PEG Solution in a 1.5-ml microtube (PEG solution should be pipetted slowly using a cut tip). Vigorously vortex tube for at least ~30 sec to ensure thorough mixing. Keep tube on ice for 30 min. Centrifuge solution in a microfuge at ~13,000 rpm for 5 min at 4°C. Harvest supernatant and store at -20°C.

Preparation of tissue/cell samples: Cell and tissue samples are deproteinized by PEG precipitation. Please follow the extraction protocol at http://www.bmrservice.com/SupplementPEG.html. Alternatively, the samples can be deproteinized by TCA precipitation (http://www.bmrservice.com/SupplementTCA.html), which is recommended for analysis of nucleotides. Some TCA-treated samples may be diluted 3-fold with dH_2O prior to assay to achieve optimal result.

α-KG Standards: Dilute the 20 mM α-KG standard 100-fold with dH_2O to 200 μM, e.g., 495 μl dH_2O + 5 μl 20 mM α-KG. Perform additional 1:1 dilution to generate 100 μM, 50 μM, and 25 μM α-KG standards. Store diluted standards at -20°C.

Preparation of working solution (Keep all thawed reagents on ice; do not over thaw solutions)

Each α-KG standard or sample requires 0.1 ml (or 0.2 ml; see step 4 below) working solution freshly prepared prior to assay. Calculate the amount of working solution needed for each assay. Keep working solution on ice and use immediately. An example of preparing 1 ml of working solution is given here:

1 ml working solution = 1 ml α-KG Assay Solution + 2 μl Ammonium (500x) + 10 μl NADPH (100x) + 20 μl GLDH (50x)

ASSAY:

1. Turn on a microplate reader or spectrophotometer. Set wavelength at 340 nm.
2. Prepare sufficient working solution as described above. Do not over thaw solutions during thawing, which can cause the solutions to lose sensitivity.
3. Pipet 20 μl of each α-KG standard and deproteinized sample to a UV-transparent 96-well plate. Alternatively, pipette 20 μl of each standard and sample to a set of 1.5-ml brown tubes if using a quartz cuvette.
4. Add 0.1 ml working solution to each well/tube followed by brief agitation or mixing. Incubate at 37°C for 60 min (do not use CO_2 incubator). For enhanced sensitivity, scale up the assay volume by mixing 40 μl standard/sample and 200 μl working solution.
5. Read O.D.340 nm. Plot the α-KG standard graph as shown on the right. A new plot must be generated for each assay.
6. Sample α-KG concentration is derived from the trend line equation of the standard plot established at the same time (x = sample α-KG concentration in μM; y = sample $O.D._{340\ nm}$).