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α-Ketoglutarate Dehydrogenase Complex (αKGDC) Assay Kit (Cat #: E-113)

COMPONENTS: αKGDC Assay Solution- 5 ml (for 100 wells); store at -80ºC **(shield solution from light during assay)**
20x αKGDC Substrate- 0.25 ml, store at -20°C or -80°C
20x Cofactors- 0.25 ml, store at -20°C or -80°C **(shield solution from light during assay)**
10x Lysis Buffer- 25 ml, store at 4ºC (dilute 10-fold with ice-cold dH_2O)
10x Extraction Buffer- 20 ml, store at 4ºC (dilute 10-fold with ice-cold dH_2O)

PRODUCT DESCRIPTION: The αKGDC enzyme activity assay is based on reduction of the tetrazolium salt INT in a NADH-coupled reaction to formazan, which exhibits an absorption maximum at 492 nm ($\varepsilon = 18\ mM^{-1}cm^{-1}$) and allows for measurement of αKGDC activity present in cell or tissue extracts. Kit components are stable for several years if stored and handled properly.

Preparation of cell/tissue lysates:

1. **Cells**: Bring up ~10^6 freeze-thawed cells in ~0.1 ml ice-cold 1x Lysis Buffer by pipetting up and down. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Collect supernatant for assays such as Hexokinase, GAPDH, and LDH. Resuspend pellet (membrane fraction) in ~50 µl ice-cold 1x Extraction Buffer by pipetting up and down thoroughly, making sure the pellet is homogenized. Leave homogenate on ice for ~10 min with agitation. Repeat homogenization. Centrifuge homogenate at ~5000 rpm for 1 min. Harvest supernatant for αKGDC assay. Recommended sample protein concentration is 0.2 - 2 mg/ml.
2. **Tissue:** Mechanically homogenize freeze-thawed tissue in ice-cold 1x Lysis Buffer (~5 mg tissue in 0.5 ml). Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Collect supernatant for assays such as Hexokinase, GAPDH, and LDH. Resuspend pellet (membrane fraction) in ~0.25 ml ice-cold 1x Extraction Buffer by pipetting up and down thoroughly, making sure the pellet is homogenized. Leave homogenate on ice for ~10 min with agitation. Repeat homogenization. Centrifuge homogenate at ~5000 rpm for 1 min. Harvest supernatant for αKGDC assay. Recommended sample protein concentration is 0.2 - 2 mg/ml.

Preparation of control solution and reaction solution

Thaw αKGDC Assay Solution, 20x αKGDC Substrate, and 20x Cofactors. Keep solutions on ice shielded from light. *Do not over thaw.* Prepare fresh control and assay solutions as follows:

Control solution is prepared by mixing 1 part of dH_2O, 1 part of 20x Cofactors, and 20 parts of αKGDC Assay Solution, e.g. 25 µl dH_2O + 25 µl 20x Cofactors + 500 µl αKGDC Assay Solution.

Reaction solution is prepared by mixing 1 part of 20x αKGDC Substrate, 1 part of 20x Cofactors, and 20 parts of αKGDC Assay Solution, e.g. 25 µl 20x αKGDC Substrate + 25 µl 20x Cofactors + 500 µl αKGDC Assay Solution.

Enzyme assay

1. Thaw protein samples and keep on ice. *Do not over thaw.* Pipette sample up and down several times prior to sample addition. Add 20 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate (for control and reaction).
2. Add 50 µl control solution to one set of sample wells and 50 µl reaction solution to the other set of sample wells. Gently agitate plate for 10 sec to mix contents. Cover plate and incubate in a 37°C incubator for 60 min or 120 min. *Do not use CO_2 incubator.*
3. Measure $O.D._{492\ nm}$ using a plate reader. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation.
4. If incubation for 60 min, αKGDC activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 70 µl/(60 min × 0.4 cm × 18 × 20 µl) = **ΔO.D. × 8.1** (or **ΔO.D. × 4.05** for 120 min). αKGDC activity can be normalized by GAPDH activity (#E-101) or protein concentration.

Additional information:

- The assay solution contains dimethyl sulfoxide (DMSO) and iodonitrotetrazolium (INT). Please refer to the product webpage or contact us for material SDS information.