Product Manual

alpha Ketoglutarate Dehydrogenase (alpha KGDHC) Assay Kit

Catalog No.: abx294007 **Revision date:** 16-Oct-17

Size: 48 tests (50 tubes)

Storage: Store reagents 1, 2 and 3 at -20 °C. Store reagents 4-10 at 4 °C before use. After preparing the Reagent 10 application solution and Working solution, use immediately or store any remaining solution at -20 °C. Avoid repeated freeze/thaw cycles.

Introduction

The alpha Ketoglutarate Dehydrogenase Assay Kit can be used to measure alpha KGDH activity in samples. Alpha KGDH is widely found in mitochondria and is a key enzyme in the regulation of the Citric Acid cycle. It catalyzes the reaction between alpha ketoglutarate, NAD^+ and Coenzyme A (CoA), producing succinyl-CoA, CO2 and NADH as products. NADH has an absorbance maxima at 340 nm, thus alpha KGDH activity can be monitored by measuring the absorbance at 340 nm.

Kit components

1. Reagent 1: 50 ml
2. Reagent 2: 10 ml
3. Reagent 3: 1 ml
4. Reagent 4: 55.5 ml
5. Reagent 5: 1 vial (lyophilized powder)
6. Reagent 6: 1 vial (lyophilized powder)
7. Reagent 7: 1 vial (lyophilized powder)
8. Reagent 8: 1 vial (lyophilized powder)
9. Reagent 9: 1 vial (lyophilized powder)
10. Reagent 10: 1 vial (lyophilized powder)

Material required but not provided

1. 37 °C / 25 °C incubator
2. Spectrophotometer (340 nm)
3. Pipette and disposable pipette tips
4. Centrifuge and vortex mixer

Reagent 10 application solution preparation

1. Add 2.1 ml of distilled water into the Reagent 10 vial. Mix fully.
2. Use immediately or store any remaining solution at -20 °C. Avoid repeated freeze/thaw cycles.

Working solution preparation

1. Transfer Reagents 5, 6, 7, 8 and 9 into Reagent 4. Mix fully.
2. Use immediately or store any remaining solution at -20 °C. Avoid repeated freeze/thaw cycles.

Sample preparation – separating plasmosin and mitochondrial proteins in tissue or cells

1. To approximately 0.1 g tissue or 5 million cells, add 1 ml of Reagent 1 and 10 µl Reagent 3. Homogenize the tissue using an ice-bath homogenizer or mortar homogenizer.
2. Centrifuge the mixture at 600 × g at 4 °C for 5 min.
3. Discard the pellet and transfer the supernatant into another tube. Centrifuge at 11000 × g at 4 °C for 10 min.
4. Extract the supernatant and transfer to a new tube. (Optional) Analyze to measure alpha KGDH activity in cytoplasm (non-mitochondrial alpha KGDH).
5. To the remaining pellet, add 200 µl of Reagent 2 and 2 µl of Reagent 3. Place the tube in an ice-bath and sonicate (200 W power, sonicate for 3 s, wait for 10 s and repeat). Analyze to measure alpha KGDH activity in mitochondria.

Procedure

1. Preheat the spectrophotometer for 30 min and set the wavelength to 340 nm. Record a zero reading using distilled water.
2. Incubate the Working solution for 5 min at 37 °C if using mammal samples, or at 25 °C for samples from other species.
3. To a cuvette, add 40 µl of Reagent 10 application solution, 60 µl of sample and then 1.1 ml of Working solution. Mix thoroughly, then measure the absorbance after 20 seconds (A_1) and then 120 seconds after measuring A_1 (A_2).

Calculation of activity by sample protein concentration

$$\alpha\ \text{KGDH activity (nmol /min /mg protein)}$$

$$= \frac{\Delta A \times V_{\text{Reaction System}} \times 10^9}{\varepsilon \times d} \times \frac{T}{V_{\text{Sample}} \times C_{\text{Protein}}}$$

$$= 1608 \times \frac{\Delta A}{C_{\text{Protein}}}$$

Calculation of activity by sample weight

$$\alpha\ \text{KGDH activity (nmol /min /g sample weight)}$$

$$= \frac{\Delta A \times V_{\text{Reaction System}} \times 10^9}{\varepsilon \times d} \times \frac{V_{\text{Extract}}}{V_{\text{Sample}} \times W \times T}$$

$$= 325 \times \frac{\Delta A}{W}$$

Calculation of activity by cell density (per 10^4 cells)

$$\alpha \text{ KGDH activity } \left(\text{nmol /min /}10^4 \text{ cells}\right)$$

$$= \frac{\Delta A \times V_{\text{Reaction System}} \times 10^9}{\varepsilon \times d} \times N \times \frac{V_{\text{Extract}}}{T}$$

$$= 0.65 \times \frac{\Delta A}{W}$$

where

ΔA	Change in absorbance between start and end of reaction	$= A_2 - A_1$
$V_{\text{Reaction System}}$	Total volume of reaction system	$= 1.2 \times 10^{-3}$ L
ε	Molar extinction coefficient of NADH	$= 6.22 \times 10^3$ L /mol /cm
d	Diameter of cuvette	= 1 cm
V_{Sample}	Volume of sample	= 0.06 ml
C_{Protein}	Sample protein concentration (in mg/ml)	
T	Reaction time	= 120 s = 2 min
W	Weight of sample (in g)	
N	Conversion factor for 5 million cells → 10^4 cells	$= 10^4 / (5 \times 10^6) = 1/500$