

Enzymatic Assay of α -KETOGLUTARATE DEHYDROGENASE

PRINCIPLE:



Abbreviations:

α -KGDH = α -Ketoglutarate Dehydrogenase

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

α -KGA = α -Ketoglutaric Acid

CoA = Coenzyme A

CONDITIONS: T = 30°C, pH 7.4, $A_{340\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 150 mM MOPS HCl Buffer, pH 7.4 at 30°C
(Prepare 100 ml in deionized water using MOPS, Sodium Salt, Prod. No. M-9381. Adjust to pH 7.4 at 30°C with 1 M HCl.)
- B. 12 mM Magnesium Chloride Solution (MgCl_2)
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Prod. No. M-0250.)
- C. 0.6 mM Calcium Chloride Solution (CaCl_2)
(Prepare 100 ml in deionized water using Calcium Chloride, Dihydrate, Prod. No. C-3881.)
- D. 18 mM Cocarboxylase Solution (Cocarboxylase)
(Prepare 10 ml in deionized water using Cocarboxylase Prod. No. C-8754.¹)
- E. 0.72 mM Coenzyme A Solution (CoA)
(Prepare 10 ml in deionized water using Coenzyme A, Sodium Salt, Prod. No. C-3144.)
- F. 20 mM β -Nicotinamide Adenine Dinucleotide, Oxidized Form (β -NAD) (Prepare 10 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Prod. No. N-7004.)

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REAGENTS: (continued)

- G. 15.6 mM L-Cysteine Solution (Cys-HCl)
(Prepare 10 ml in deionized water using L-Cysteine, Hydrochloride, Monohydrate, Prod. No. C-7880. Adjust to pH 7.4 at 30°C with 1 M NaOH.)
- H. 75 mM α -Ketoglutaric Acid Solution (α -KGA)
(Prepare 10 ml in deionized water using α -Ketoglutaric Acid, Monosodium Salt, Prod. No. K-1875.)
- I. 50 mM MOPS HCl Buffer, pH 7.4 at 30°C (Enz Dil)
(Prepare 100 ml in deionized water using MOPS, Sodium Salt, Sigma Prod. No. M-9381. Adjust to pH 7.4 at 30°C with 1 M HCl.)
- J. α -Ketoglutarate Dehydrogenase Enzyme Solution (α -KGDH)
(Immediately before use, prepare a solution containing 0.5 - 1.0 units/ml of α -Ketoglutarate Dehydrogenase in cold Reagent I.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.00	1.00
Reagent B (MgCl ₂)	0.05	0.05
Reagent C (CaCl ₂)	0.05	0.05
Reagent D (Coccarboxylase)	0.05	0.05
Reagent E (Coenzyme A)	0.50	0.50
Reagent F (β -NAD)	0.30	0.30
Reagent G (Cys-HCl)	0.50	0.50
Reagent I (α -KGDH)	0.05	0.05
Deionized Water	0.30	0.30

Mix by inversion and equilibrate to 30°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent H (α -KGA)	0.20	-----
Deionized Water	-----	0.20

Immediately mix by inversion and record the increase in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{340\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

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CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(6.22)(0.05)}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β -NADH at 340 nm

0.05 = Volume (in milliliter) of enzyme used

UNIT DEFINITION:

One unit will convert 1.0 μ mole of β -NAD to β -NADH per minute at pH 7.4 at 30°C in the presence of saturating levels of coenzyme A.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 50.8 mM MOPS, 0.2 mM MgCl_2 , 0.01 mM CaCl_2 , 0.3 mM cocarboxylase, 0.12 mM coenzyme A, 2.0 mM β -nicotinamide adenine dinucleotide, 2.6 mM L-cysteine, 5.0 mM a-ketoglutaric acid, and 0.025 - 0.05 units a-ketoglutarate dehydrogenase.

NOTES:

1. Cocarboxylase is also known as thiamine pyrophosphate.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.