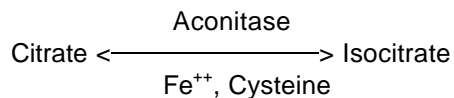


**Enzymatic Assay of ACONITASE
(EC 4.2.1.3)**

PRINCIPLE:



Abbreviations:

β -NADP = β -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form

β -NADPH = β -Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

ICD = Isocitric Dehydrogenase

CONDITIONS: T = 25°C, pH = 7.4, $A_{340\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Tris Buffer, pH 7.4 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.4 at 25°C with 1 M HCl.)
- B. 2 mM Citric Acid Solution (Cit)
(Prepare 20 ml in deionized water using Citric Acid Free Acid, Monohydrate, Sigma Prod. No. C-7129. Adjust to pH 7.4 with 100 mM NaOH.)
- C. 5.4 mM β -Nicotinamide Adenine Dinucleotide Phosphate Solution (β -NADP)
(Dissolve the contents of a 5 mg vial of β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock No. 240-305, in the appropriate volume of deionized water **or** prepare 1 ml in deionized water using β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505. **PREPARE FRESH.**)

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REAGENTS: (continued)

- D. 1 mM Ferrous Ammonium Sulfate Solution ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$)
(Prepare 1 ml in deionized water using Ferrous Ammonium Sulfate, Hexahydrate, Sigma Prod. No. F-3754. **PREPARE FRESH.**)
- E. 20 mM Manganese Sulfate (MnSO_4)
(Prepare 2 ml in deionized water using Manganese Sulfate, Sigma Prod. No. M-7634.)
- F. 50 mM L-Cysteine Solution, pH 7.4 at 25°C (Cys)
(Prepare 5 ml in deionized using L-Cysteine Hydrochloride, Sigma Prod. No. C-7880. Adjust to pH 7.4 at 25°C with 1 M NaOH. **PREPARE FRESH.**)
- G. Activation Buffer
(Prepare by combining 4 ml of Reagent A, 0.10 ml of Reagent D, and 0.2 ml of Reagent F. Store at 0°C.)
- H. Isocitric Dehydrogenase Enzyme Solution (IsoDH)
(Immediately before use, prepare a solution containing 14 units/ml in deionized water using Isocitric Dehydrogenase, Sigma Prod. No. I-2516.)
- I. Aconitase Enzyme
(Use 15 mg.)

PROCEDURE:

Prepare activated Aconitase enzyme solution by combining the following reagents in a suitable vial:

| | |
|-------------------------------|---------|
| Reagent I (Enzyme) | 15 mg |
| Reagent G (Activation Buffer) | 2.15 ml |

Mix and incubate at 0°C for 1 hour.

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

| | <u>Test</u> | <u>Blank</u> |
|-------------------------------|-------------|--------------|
| Deionized Water | 1.45 | 1.45 |
| Reagent A (Buffer) | 1.00 | 1.00 |
| Reagent B (Cit) | 0.10 | 0.10 |
| Reagent C (β -NADP) | 0.10 | 0.10 |
| Reagent E (MnSO_4) | 0.20 | 0.20 |
| Reagent H (IsoDH) | 0.05 | 0.05 |

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PROCEDURE: (continued)

Mix by inversion and equilibrate at 25°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer.

Then add:

| | <u>Test</u> | <u>Blank</u> |
|-------------------------------------|-------------|--------------|
| Activated Aconitase Enzyme Solution | 0.10 | ----- |
| Reagent G (Activation Buffer) | ----- | 0.10 |

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/g solid} = \frac{\text{units/ml enzyme (1000)}}{\text{mg/ml enzyme}}$$

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

UNIT DEFINITION:

One unit will convert 1.0 μmole of citrate (via cis- α -aconitate) to isocitrate per minute at pH 7.4 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 36 mM Tris, 0.07 mM citric acid, 0.18 mM β -nicotinamide adenine dinucleotide phosphate, 1.3 mM manganese sulfate, 0.0008 mM ferrous ammonium sulfate, 0.08 mM L-cysteine, 0.7 unit isocitric dehydrogenase, and 0.70 mg aconitase.

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REFERENCE:

Morrison, J.F. (1954) *Biochemical Journal* **58**, 685-692

NOTES:

1. This assay is based on the cited reference.
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.