



SIGMA QUALITY CONTROL TEST PROCEDURE

Product Information

Enzymatic Assay of ALCOHOL DEHYDROGENASE, NADP⁺ DEPENDENT (EC 1.1.1.2)

PRINCIPLE:

2-Propanol + β -NADP Alcohol Dehydrogenase > Acetone + β -NADPH

Abbreviations used:

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

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

CONDITIONS: T = 40°C, pH = 7.8, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Tris HCl Buffer, pH 7.8 at 40°C
(Prepare 100 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253.
Adjust to pH 7.8 at 40°C with 1 M NaOH.)
- B. 15 mM β -Nicotinamide Adenine Dinucleotide Phosphate Solution (β -NADP)
(Prepare 2.5 ml in Reagent A using β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505 or dissolve the contents of one 10 mg vial of β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Stock No. 240-310, in an appropriate volume of Reagent A. **PREPARE FRESH.**)
- C. 1.5 M 2-Propanol Solution (2-Prop)
(Prepare 10 ml in Reagent A using Isopropanol, Anhydrous, Sigma Stock No. 405-7.)
- D. Alcohol Dehydrogenase, NADP⁺ Dependent Enzyme Solution
(Immediately before use, prepare a solution containing 0.10 - 0.40 unit/ml of Alcohol Dehydrogenase, NADP⁺ Dependent in cold Reagent A.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.40	2.60
Reagent B (β-NADP)	0.10	0.10
Reagent C (2-Prop)	0.30	0.30

Mix by inversion and equilibrate to 40°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.20	-----
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Immediately mix by inversion and record the increase in A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(6.22)(0.2)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.2 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will oxidize 1.0 μmole of 2-propanol to acetone per minute at pH 7.8 at 40°C in the presence of β-NADP⁺.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 100 mM Tris, 0.5 mM β -nicotinamide adenine dinucleotide phosphate, 150 mM 2-propanol and 0.02 - 0.08 unit alcohol dehydrogenase, β -NADP⁺ dependent.

REFERENCES:

Lamed, R.J., and Zeikus, J.G. (1980) *Journal of Bacteriology* **141**, 1251-1257

Lamed, R.J., Keinan, E., and Zeikus, J.G. (1981) *Enzyme Microbial Technology* **3**, 144-148

Lamed, R.J., and Zeikus, J.G. (1981) *Biochemical Journal* **195**, 183-190

NOTES:

1. This assay is based on the cited references.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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