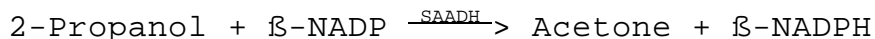


**Enzymatic Assay of (S)-AROMATIC ALCOHOL DEHYDROGENASE,
NADP⁺ Dependent
(EC 1.1.1.2)**

PRINCIPLE:



Abbreviations used:

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

SAADH = (S)-Aromatic Alcohol Dehydrogenase, NADP⁺ dependent

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

CONDITIONS: T = 50°C, pH 9.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Glycine Buffer with 2.0 mM DL-Dithiothreitol, pH 9.0 at 50°C
(Prepare 100 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126, and DL-Dithiothreitol, Sigma Prod. No. D-0632. Adjust to pH 9.0 at 50°C with 1 M NaOH.)
- B. 26 mM β -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form Solution (β -NADP)
(Dissolve the contents of one 10 mg vial of β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Preweighed Vial, Sigma Stock No. 240-310, in the appropriate volume of deionized water.)
- C. 650 mM 2-Propanol Solution (2-Prop)
(Prepare 1 ml in deionized water using Isopropanol, Anhydrous, Sigma Stock No. 405-7.)
- D. S-Aromatic Alcohol Dehydrogenase, NADP⁺ Dependent Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of S-Aromatic Alcohol Dehydrogenase, NADP⁺ Dependent in cold deionized water.)

**Enzymatic Assay of (S)-AROMATIC ALCOHOL DEHYDROGENASE,
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PROCEDURE:

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

	Test	Blank
Reagent D (Enzyme Solution)	0.02	-----
Deionized Water	-----	0.02
Reagent A (Buffer)	1.00	1.00
Reagent C (2-Prop)	0.10	0.10

Mix by inversion and equilibrate to 50°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent B (β -NADP)	0.10	0.10
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Immediately mix by inversion and record the increase in A_{340nm} for approximately 5 minutes. Obtain the $r A_{340nm}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{340nm}/\text{min Test} - r A_{340nm}/\text{min Blank})(1.22)(df)}{(6.22)(0.02)}$$

1.22 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.02 = 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 9.0 at 50°C.

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FINAL ASSAY CONCENTRATIONS:

In a 1.22 ml reaction mix, the final concentrations are 41 mM glycine, 1.6 mM DL-dithiothreitol, 2.1 mM β -nicotinamide adenine dinucleotide phosphate, 53 mM 2-propanol, and 0.01 - 0.02 unit (S)-aromatic alcohol dehydrogenase, NADP⁺ dependent.

REFERENCE:

Bryant, F. and Ljungdahl, L.G. (1981) *Biochemical and Biophysical Research Communications* **100**, 793-799

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.