Creative Enzymes

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

PRINCIPLE:

2-Propanol + ß-NADP $\xrightarrow{\text{SAADH}}$ Acetone + ß-NADPH

Abbreviations used:
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
SAADH = (S)-Aromatic Alcohol Dehydrogenase, $NADP^+$ dependent
ß-NADPH = ß-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, and DL-Dithiothreitol, Adjust to pH 9.0 at 50°C with 1 M NaOH.)

B. 26 mM ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form Solution (ß-NADP)
(Dissolve the contents of one 10 mg vial of ß-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Preweighed Vial, in the appropriate volume of deionized water.)

C. 650 mM 2-Propanol Solution (2-Prop)
(Prepare 1 ml in deionized water using Isopropanol, Anhydrous)

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.)

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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 (ß-NADP)	0.10	0.10

Immediately mix by inversion and record the increase in A_{340nm} for approximately 5 minutes. Obtain the $r\ A_{340nm}$/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 ß-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 ß-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 our Product or Stock numbers are specified, equivalent reagents may be substituted.

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

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