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

PRINCIPLE:

$$\text{2-Hexanol} + \text{ß-NADP} \xrightarrow[Mg^{2+}]{\text{RAADH}} \text{2-Hexanone} + \text{ß-NADPH}$$

Abbreviations used:
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
RAADH = (R)-Aromatic Alcohol Dehydrogenase
ß-NADPH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

CONDITIONS: T = 50°C, pH = 7.8, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM HEPES Buffer with 2.0 mM Magnesium Chloride and 2.0 mM Dithiothreitol, pH 7.8 at 50°C
(Prepare 100 ml in deionized water using HEPES, Free Acid Sigma Prod. No. H-3375, Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250, and DL-Dithiothreitol, Sigma Prod. No. D-0632. Adjust to pH 7.8 at 50°C with 1 M NaOH. **PREPARE FRESH**.)

B. 26 mM ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form (ß-NADP)
(Prepare 1 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-3886.)

C. 100 mM 2-Hexanol Solution (2-Hex)
(Prepare 5 ml in deionized water using 2-Hexanol, Aldrich Stock No. 12,857-0.)

D. (R)-Aromatic Alcohol Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 1.66 - 3.32 unit/ml of (R)-Aromatic Alcohol Dehydrogenase, $NADP^+$ Dependent in cold deionized water.)

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PROCEDURE:

Equilibrate Reagent A (Buffer) and the sample cell of the spectrophotometer to 50°C.

Pipette (in milliliters) the following reagents into suitable cuvettes by adding the reagents as a bead of liquid adhering to the side of the cuvette:

	Test	Blank
Reagent D (Enzyme Solution)	0.01	------
Deionized Water	------	0.01

Then add:

Reagent A (Buffer)	1.00	1.00

Direct the flow from the pipette onto the bead of Reagent D to ensure effective mixing. Then add:

Reagent B (ß-NADP)	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 C (2-Hex)	0.10	0.10

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:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(1.21)(df)}{(6.22)(0.01)}$$

1.21 = Volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADPH at 340 nm
0.01 = Volume (in milliliter) of enzyme used

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

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CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of 2-hexanol to 2-hexanone per minute at pH 7.8 at 50°C.

FINAL ASSAY CONCENTRATION:

In a 1.21 ml reaction mix, the final concentration are 41 mM HEPES, 1.6 mM magnesium chloride, 1.6 mM DL - dithiothreitol, 2.1 mM ß-nicotinamide adenine dinucleotide phosphate, 8.3 mM 2-hexanol, and 0.0166 - 0.0332 unit (R)-aromatic alcohol dehydrogenase.

REFERENCES:

Hummel, W. (1990) *Applied Microbiology and Biotechnology* **34**, 15-19

Bradshaw, C.W., Hummel, W. and Wong, C.-H. (1992) *Journal of Organic Chemistry* **57**, 1532-1536

NOTES:

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