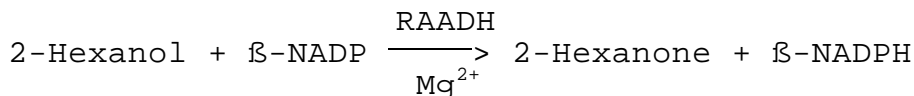


**Enzymatic Assay of (R)-AROMATIC ALCOHOL DEHYDROGENASE,
NADP⁺ Dependent
(EC 1.1.1.2)**

PRINCIPLE:



Abbreviations used:

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

RAADH = (R)-Aromatic Alcohol Dehydrogenase

β -NADPH = β -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 β -Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form (β -NADP)
(Prepare 1 ml in deionized water using β -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:

	<u>Test</u>	<u>Blank</u>
Reagent D (Enzyme Solution)	0.01	-----
Deionized Water	-----	0.01

Then add:

Reagent A (Buffer)	1.00	1.00
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Direct the flow from the pipette onto the bead of Reagent D to ensure effective mixing. Then add:

Reagent B (β-NADP)	0.10	0.10
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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
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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:

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

1.21 = Volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β-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 μ mole 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 β -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.

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