3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

# ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

**Enzymatic Assay of SORBITOL DEHYDROGENASE**
**(EC 1.1.1.14)**

**PRINCIPLE:**

$$\text{D-Fructose} + \beta\text{-NADH} \xrightarrow{\text{Sorbitol Dehydrogenase}} \text{D-Sorbitol} + \beta\text{-NAD}$$

Abbreviations used:
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

**CONDITIONS:** T = 25°C, pH 7.6, $A_{340nm}$, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 100 mM Triethanolamine Buffer, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Triethanolamine, Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.6 at 25°C with 5 M NaOH.)

B. 1.1 M D-Fructose Solution (Fructose)
(Prepare 5 ml in deionized water using D(-)Fructose, Sigma Prod. No. F-0127.)

C. 12.8 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form, Solution (β-NADH)
(Dissolve the contents of one 10 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Preweighed Vial, Disodium Salt, Sigma Stock No. 340-110, in the appropriate volume of deionized water. **PREPARE FRESH**.)

D. 1.0% (w/v) Bovine Serum Albumin (BSA)
(Prepare 10 ml in deionized water using Albumin, Bovine, Sigma Prod. No. A-6003.)

E. Sorbitol Dehydrogenase Enzyme Solution
(Prepare a solution containing 70 - 150 units/ml of Sorbitol Dehydrogenase in cold deionized water. Store at 4°C for 1 hour. Immediately before use, dilute to a final concentration of 0.55 - 0.75 unit/ml with cold Reagent D.)

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**PROCEDURE:**

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

| | Test | Blank |
|---|---|---|
| Reagent A (Buffer) | 2.35 | 2.35 |
| Reagent B (Fructose) | 0.50 | 0.50 |
| Reagent C (β-NADH) | 0.05 | 0.05 |

Mix by inversion and equilibrate to 25°C. Monitor the $A_{340nm}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

| | | |
|---|---|---|
| Reagent E (Enzyme Solution) | 0.10 | ------ |
| Reagent D (BSA) | ------ | 0.10 |

Immediately mix by inversion and record the decrease in $A_{340nm}$ for approximately 5 minutes. Obtain the $\Delta A_{340nm}$/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3)(df)}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADH at 340nm
0.1 = Volume (in milliliter) of enzyme

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

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

**UNIT DEFINITION:**

One unit will convert 1.0 μmole of D-fructose to D-sorbitol per minute at pH 7.6 at 25°C.

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**FINAL ASSAY CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 78 mM triethanolamine, 183 mM D-fructose, 0.2 mM β-nicotinamide adenine dinucleotide, reduced form, 0.033% (w/v) bovine serum albumin and 0.055 - 0.075 unit sorbitol dehydrogenase.

**REFERENCE:**

Gerlach, U. and Hiby, W. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 2nd ed., Volume II, 569-573, Academic Press Inc., New York, NY

**NOTES:**

1. This assay is based on the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.