Enzymatic Assay of ß-GALACTOSE DEHYDROGENASE
(EC 1.1.1.48)

PRINCIPLE:

ß-D-Galactose + ß-NAD $\xrightarrow{\text{ß-Galactose Dehydrogenase}}$ D-Galactonate + ß-NADH

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

CONDITIONS: T = 25°C, pH = 8.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 8.6 at 25°C
(Prepare 50 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 8.6 at 25°C with 1 M HCl.)

B. 72 mM ß-Nicotinamide Adenine Dinucleotide Solution (ß-NAD)
(Dissolve the contents of one 50 mg vial of ß-Nicotinamide Adenine Dinucleotide, Stock No. 260-150, in the appropriate volume of deionized water. **PREPARE FRESH.**)

C. 10% (w/v) D(+)Galactose Solution (Gal)
(Prepare 1 ml in deionized water using D(+)Galactose, Prod. No. G-0750.)[1]

D. 1 M Ammonium Sulfate (Enzyme Diluent)
(Prepare 10 ml in deionized water using Ammonium Sulfate, Prod. No. A-4915.)

E. ß-Galactose Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.15 - 0.30 unit/ml of ß-Galactose Dehydrogenase in cold Reagent D.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	3.00	3.00
Reagent B (ß-NAD)	0.10	0.10
Reagent C (Gal)	0.10	0.10

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

	Test	Blank
Reagent D (Enzyme Diluent)	------	0.10
Reagent E (Enzyme Solution)	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:

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{340nm}/\text{min Test} - \text{r } A_{340nm}/\text{min Blank})(3.3)(df)}{(6.22)(0.1)}$$

3.3 = Volume (in milliliters) of assay
df = Dilution factor
0.1 = Volume (in milliliter) of enzyme used
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
RM = Reaction Mix

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

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of D-galactose to D-galactonate per minute at pH 8.6 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.30 ml reaction mix, the final concentrations are 91 mM Tris, 2.2 mM ß-nicotinamide adenine dinucleotide, 0.30% (w/v) D-galactose, 30 mM ammonium sulfate and 0.015 - 0.030 unit ß-galactose dehydrogenase.

REFERENCES:

Bergmeyer, H.U., (1974) *Methods of Enzymatic Analysis*, 2nd ed., Volume 1, 453-454.

NOTES:

1. Mutorotation of galactose does not affect the activity of this enzyme assay.

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

3. This assay is based on the cited reference.

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