Enzymatic Assay of GLYCEROL DEHYDROGENASE
(EC 1.1.1.6)

PRINCIPLE:

Glycerol + ß-NAD $\xrightarrow{\text{Glycerol Dehydrogenase}}$ Dihydroxyacetone + 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 10.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 500 mM Potassium Bicarbonate Solution ($KHCO_3$)
(Prepare 100 ml in deionized water using Potassium Bicarbonate, Sigma Prod. No. P-9144.)

B. 500 mM Potassium Carbonate Buffer, pH 10.0 at 25°C
(Prepare 100 ml in deionized water using Potassium Carbonate, Sigma Prod. No. P-4379. Adjust to pH 10.0 at 25°C with Reagent A ($KHCO_3$).)

C. 1 M Ammonium Sulfate Solution ($(NH_4)_2SO_4$)
(Prepare 10 ml in deionized water using Ammonium Sulfate, Sigma Prod. No. A-5132.)

D. 10 mM ß-Nicotinamide Adenine Dinucleotide, Oxidized Form, Solution (ß-NAD)
(Prepare 2 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004, or dissolve the contents of one 50 mg vial of ß-Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-150, in the appropriate volume of deionized water. **PREPARE FRESH.**)

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REAGENTS: (continued)

E. 1 M Glycerol Solution (Glycerol)
(Prepare 100 ml in deionized water using Glycerol, Sigma Prod. No. G-9012.)

F. 50 mM Potassium Phosphate with 0.1% (w/v) Bovine Serum Albumin and 0.05 mM Manganese Chloride Solution, pH 7.5 at 25°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, Albumin, Bovine, Sigma Prod. No. A-4503, or equivalent and Manganese Chloride, Tetrahydrate, Sigma Prod. M-3634. Adjust to pH 7.5 at 25°C with 1 M KOH.)[1]

G. Glycerol Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 - 0.6 unit/ml of Glycerol Dehydrogenase in cold Reagent F.)

PROCEDURE:

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

	Test	Blank
Deionized Water	1.80	1.80
Reagent B (Buffer)	0.60	0.60
Reagent C ($(NH_4)_2SO_4$)	0.10	0.10
Reagent D (ß-NAD)	0.10	0.10
Reagent E (Glycerol)	0.30	0.30

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

Reagent F (Enzyme Diluent)	------	0.10
Reagent G (Enzyme Solution)	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.

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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 ß-NADH at 340 nm
0.1 = 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 glycerol to dihydroxyacetone per minute at pH 10.0 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 100 mM potassium carbonate, 33 mM ammonium sulfate, 0.33 mM ß-nicotinamide adenine dinucleotide, 100 mM glycerol, 1.7 mM potassium phosphate, 0.003% (w/v) bovine serum albumin, 0.002 mM manganese chloride and 0.02 - 0.06 unit glycerol dehydrogenase.

NOTES:

1. Do not use if solution becomes light brown or hazy light brown.

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