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 8.8, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer with 0.1% (w/v) Bovine Serum Albumin, pH 8.8 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379, and Albumin Bovine, Prod. No. A-4503 or equivalent. Adjust to pH 8.8 at 25°C with 1 M KOH.)

B. 2565 mM Glycerol with 100 mM Glycine, 100 mM Potassium Chloride Solution, pH 8.8 at 25°C (Glycerol)
(Prepare 100 ml in deionized water using Glycerol, Prod. No. G-9012, Glycine Free Base, Prod. No. G-7126, and Potassium Chloride, Prod. No. P-4504. Adjust to pH 8.8 at 25°C with 1 M NaOH.)

C. 52 mM ß-Nicotinamide Adenine Dinucleotide Solution (ß-NAD)
(Prepare 2 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Prod. No. N-7004 or 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.**)

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REAGENTS: (continued)

D. Glycerol Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.35 - 0.70 unit/ml of Glycerol Dehydrogenase in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent B (Glycerol)	2.50	2.50
Reagent C (ß-NAD)	0.15	0.15

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

Reagent A (Buffer)	------	0.05
Reagent D (Enzyme Solution)		0.05

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:

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

6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
RM = Reaction Mix

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of glycerol to dihydroxyacetone per minute at pH 8.8 at 25°C.

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FINAL ASSAY CONCENTRATIONS:

In a 2.70 ml reaction mix, the final concentrations are 93 mM glycine, 93 mM potassium chloride, 2375 mM glycerol, 2.9 mM ß-NAD, 2 mM potassium phosphate, 0.002% (w/v) BSA and 0.018 - 0.035 unit glycerol dehydrogenase.

REFERENCE:

Burton, R. M. (1955) *Methods in Enzymology*, Volume I, 397.

NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.