

**Enzymatic Assay of GLYCEROL DEHYDROGENASE  
(EC 1.1.1.6)**

**PRINCIPLE:**

Glycerol +  $\beta$ -NAD Glycerol Dehydrogenase > Dihydroxyacetone +  $\beta$ -NADH

Abbreviations used:

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide,  
Oxidized Form

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide,  
Reduced Form

**CONDITIONS:** T = 25°C, pH 10.0, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 500 mM Potassium Bicarbonate Solution (KHCO<sub>3</sub>)  
(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<sub>3</sub>).)
- C. 1 M Ammonium Sulfate Solution ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>)  
(Prepare 10 ml in deionized water using Ammonium Sulfate, Sigma Prod. No. A-5132.)
- D. 10 mM  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form, Solution ( $\beta$ -NAD)  
(Prepare 2 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004, or dissolve the contents of one 50 mg vial of  $\beta$ -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.)<sup>1</sup>
- 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:

	<u>Test</u>	<u>Blank</u>
Deionized Water	1.80	1.80
Reagent B (Buffer)	0.60	0.60
Reagent C ((NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> )	0.10	0.10
Reagent D (β-NAD)	0.10	0.10
Reagent E (Glycerol)	0.30	0.30

Mix by inversion and equilibrate to 25°C. Monitor the A<sub>340nm</sub> 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<sub>340nm</sub> for approximately 5 minutes. Obtain the r A<sub>340nm</sub>/minute using the maximum linear rate for both the Test and Blank.

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**CALCULATIONS:**

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -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  $\mu$ mole 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  $\beta$ -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.

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