

**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 8.8, A<sub>340nm</sub>, 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  $\beta$ -Nicotinamide Adenine Dinucleotide Solution ( $\beta$ -NAD)  
(Prepare 2 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide, Prod. No. N-7004 or dissolve the contents of one 50 mg vial of  $\beta$ -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:

	<u>Test</u>	<u>Blank</u>
Reagent B (Glycerol)	2.50	2.50
Reagent C (β-NAD)	0.15	0.15

Mix by inversion and equilibrate to 25°C. Monitor the  
A<sub>340nm</sub> 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<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.

**CALCULATIONS:**

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

6.22 = Millimolar extinction coefficient of β-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  $\beta$ -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.

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