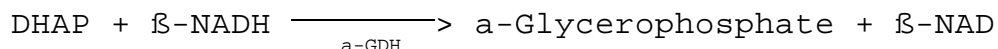
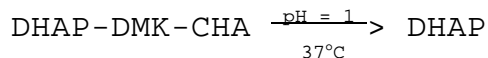


**Determination of the  
Concentration and Molecular Weight of  
DIHYDROXYACETONE PHOSPHATE DIMETHYLKETAL**

**PRINCIPLE:**



Abbreviations used:

DHAP-DMK-CHA = Dihydroxyacetone Phosphate Dimethylketal,  
Di(Monocyclohexylammonium)

DHAP = Dihydroxyacetone Phosphate

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

a-GDH = a-Glycerophosphate Dehydrogenase

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

**CONDITIONS:** T = 25°C, pH = 7.6, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Spectrophotometric

**REAGENTS:**

- A. 200 mM Triethanolamine HCl Buffer, pH 7.6 at 25°C  
(Prepare 100 ml in deionized water using  
Triethanolamine Hydrochloride, Sigma Prod. No. T-1502.  
Adjust to pH 7.6 at 25°C with 1 M NaOH.)
- B. 3.8 mM  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced  
Form ( $\beta$ -NADH)  
(Prepare 1 in Reagent A using  $\beta$ -Nicotinamide Adenine  
Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod.  
No. N-8129.)
- C. Dihydroxyacetone Phosphate Solution (DHAP)  
(Weigh approximately 1.25 mg of Dihydroxyacetone  
Phosphate Dimethylketal, Di(monocyclohexylammonium  
Salt:Monohydrate) and dissolve in 5 ml of 0.1 N HCl.  
Incubate at 37°C for 1.5 - 2.0 hours. Then dilute a  
1.0 ml aliquot to 5 ml with deionized water.)

**Determination of the  
Concentration and Molecular Weight of  
DIHYDROXYACETONE PHOSPHATE DIMETHYLKETAL**

**REAGENTS:** (continued)

- D. a-Glycerophosphate Dehydrogenase Enzyme Solution (a-GDH)  
(Immediately before use, prepare a solution containing approximately 50 units/ml of a-Glycerophosphate Dehydrogenase, Sigma Prod. No. G-6751, in Reagent A.)

**PROCEDURE:**

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

	Test	Blank
Reagent A (Buffer)	1.90	2.00
Reagent B (β-NADH)	0.10	-----
Reagent C (DHAP)	1.00	1.00

Mix by inversion and equilibrate at 25°C for 5 minutes. Record the initial  $A_{340\text{nm}}$  using a suitably thermostatted spectrophotometer. Then add:

Reagent D (a-GDH)	0.02	0.02
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Mix by inversion and allow the reaction to proceed for 5-10 minutes. Record the final  $A_{340\text{nm}}$  for both the Test and Blank.

**CALCULATIONS:**

$$r A = A_i \times \frac{3}{3.02} - A_f$$

change

$A_i$  = Initial absorbance  
 $A_f$  = Final Absorbance  
 3/3.02 = Correction for volume

$$\text{Micromoles DHAP/weighed sample} = \frac{(r A \text{ Test} - r A \text{ Blank})(3.02)(25)}{6.22}$$

3.02 = Total volume (in milliliters) of assay  
 25 = Dilution factor  
 6.22 = Millimolar extinction coefficient of β-NADH at 340 nm

mg sample weighed x 1000

Apparent molecular weight =  $\frac{\text{-----}}{\mu\text{moles DHAP/weighed sample}}$

**Determination of the  
Concentration and Molecular Weight of  
DIHYDROXYACETONE PHOSPHATE DIMETHYLKETAL**

**CALCULATIONS:** (continued)

1000 = Conversion factor from mg to  $\mu$ g  
DHAP = Dihydroxyacetone phosphate

**FINAL ASSAY CONCENTRATION:**

In a 3.02 ml reaction mix, the final concentrations are 134 mM triethanolamine, 0.13 mM  $\beta$ -nicotinamide adenine dinucleotide, reduced form, and 1 unit  $\alpha$ -glycerophosphate dehydrogenase.

**REFERENCE:**

Bücher, T. and Hohorst, H.-J. (1965) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U., ed.) First edition, 246-252, Academic Press, New York, NY

**NOTES:**

1. This assay is based on the cited reference.
2.  $\alpha$ -Glycerophosphate Dehydrogenase Unit Definition:  
One unit will convert 1.0  $\mu$ mole of dihydroxyacetone phosphate to  $\alpha$ -glycerophosphate per minute at pH 7.4 at 25°C.
3. 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.**