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ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of ALDOLASE[1]
(EC 4.1.2.13)

PRINCIPLE:

Fructose 1,6-Diphosphate + H_2O $\xrightarrow{\text{Aldolase}}$ G 3-P + DHAP

G 3-P $\xrightarrow{\text{TPI}}$ DHAP

2 DHAP + 2 β-NADH $\xrightarrow{\text{α-GDH}}$ 2 α-Glycerophosphate + 2 β-NAD

Abbreviations used:
G 3-P = Glyceraldehyde 3-Phosphate
DHAP = Dihydroxyacetone Phosphate
TPI = Triosephosphate Isomerase
α-GDH = α-Glycerophosphate Dehydrogenase
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: T = 25°C, pH = 7.4, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 7.4 at 25°C.
(Prepare 250 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.4 at 25°C with 1 M HCl.)

B. 58 mM Fructose 1,6-Diphosphate Solution (F 1,6-DP)
(Prepare 1 ml in deionized water using D-Fructose 1,6-Diphosphate, Tetra(cyclohexylammonium) Salt, Sigma Prod. No. F-0752.)

C. 4.0 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)
(Prepare 2 ml in cold deionized water using β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129 or dissolve the contents of one 5 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock. No. 340-105, in the appropriate volume of deionized water.)

D. α-Glycerophosphate Dehydrogenase/Triosephosphate Isomerase Enzyme Solution (α-GDH/TPI)
(Immediately before use, prepare a solution containing 50 α-GDH units/ml of α-Glycerophosphate Dehydrogenase/Triosephosphate Isomerase, Sigma Prod. No. G-6755, in cold deionized water.)

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REAGENTS: (continued)

E. Aldolase Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 - 0.50 unit/ml of Aldolase in cold Reagent A.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.60	2.60
Reagent B (F 1,6-DP)	0.10	0.10
Reagent C (β-NADH)	0.10	0.10
Reagent D (α-GDH/TPI)	0.10	0.10

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

Reagent A (Buffer)	------	0.10
Reagent E (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/min using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3)(df)}{(2)(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
2 = 2 moles of β-NADH converted to 2 moles of β-NAD per mole of Fructose 1,6-Diphosphate
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm
0.1 = Volume (in milliliter) of enzyme used

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CALCULATIONS: (continued)

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will convert 1.0 µmole of fructose 1,6-diphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate per minute at pH 7.4 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 90 mM Tris, 1.9 mM fructose 1,6-diphosphate, 0.13 mM β-nicotinamide adenine dinucleotide, 5 units α-glycerophosphate dehydrogenase/triosephosphate isomerase (based on α-glycerophosphate dehydrogenase units) and 0.025 - 0.050 unit aldolase.

REFERENCE:

Bergmeyer, H.U. (1974) *Methods of Enzymatic Analysis*, Second Edition, Volume I, 430

NOTES:

1. This enzyme assay is not to be used to assay Aldolase, from Staphylococcus aureus, Sigma Prod. No. A-2548, Aldolase, insoluble enzyme attached to polyacrylamide from Rabbit Muscle, Sigma Prod. No. A-1386, and Aldolase from Baker's Yeast, Sigma Prod. No. A-9562.

2. α-Glycerophosphate Dehydrogenase Unit Definition: One unit will convert 1.0 µmole of dihydroxyacetone phosphate to α-glycerophosphate per minute at pH 7.4 at 25°C.

3. Triosephosphate Isomerase Unit Definition: One unit will convert 1.0 µmole of D-glyceraldehyde 3-phosphate to dihydroxyacetone phosphate per minute at pH 7.6 at 25°C.

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