



## SIGMA QUALITY CONTROL TEST PROCEDURE

## Product Information

### Enzymatic Assay of ALDOLASE<sup>1</sup> (EC 4.1.2.13)

#### PRINCIPLE:

Fructose 1,6-Diphosphate + H<sub>2</sub>O  $\xrightarrow{\text{Aldolase}}$  G 3-P + DHAP

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

2 DHAP + 2 β-NADH  $\xrightarrow{\alpha\text{-GDH}}$  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<sub>340nm</sub>, 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.)

### Enzymatic Assay of ALDOLASE<sup>1</sup> (EC 4.1.2.13)

#### 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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.60	2.60
Reagent B (F 1,6-DP)	0.10	0.10
Reagent C ( $\beta$ -NADH)	0.10	0.10
Reagent D ( $\alpha$ -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  $\Delta A_{340nm}/min$  using the maximum linear rate for both the Test and Blank.

# **CALCULATIONS:**

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

2 = 2 moles of  $\beta$ -NADH converted to 2 moles of  $\beta$ -NAD per mole of Fructose 1,6-Diphosphate

6.22 = Millimolar extinction coefficient of  $\beta$ -NADH at 340 nm

0.1 = Volume (in milliliter) of enzyme used

## Enzymatic Assay of ALDOLASE<sup>1</sup> (EC 4.1.2.13)

### 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  $\mu$ mole 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  $\beta$ -nicotinamide adenine dinucleotide, 5 units  $\alpha$ -glycerophosphate dehydrogenase/triosephosphate isomerase (based on  $\alpha$ -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.  $\alpha$ -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. Triosephosphate Isomerase Unit Definition: One unit will convert 1.0  $\mu$ mole 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.

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