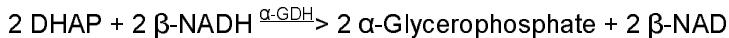
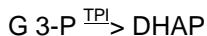
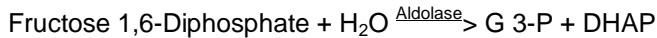

SIGMA QUALITY CONTROL TEST PROCEDURE**Product Information****Enzymatic Assay of ALDOLASE¹
(EC 4.1.2.13)****PRINCIPLE:**

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.)

**Enzymatic Assay of ALDOLASE¹
(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 (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 $A_{340\text{nm}}$ 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 $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{340\text{nm}}/\text{min}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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

Enzymatic Assay of ALDOLASE¹ (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 μ 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 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 μ mole 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 μ 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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