



## Product Information

### SIGMA QUALITY CONTROL TEST PROCEDURE

#### Enzymatic Assay of GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE<sup>1</sup> (EC 1.2.1.12)

##### PRINCIPLE:



##### Abbreviations used:

3-PGA = 3-Phosphoglyceric Acid

ATP = Adenosine 5'-Triphosphate

3-PGK = 3-Phosphoglyceric Phosphokinase

ADP = Adenosine 5'-Diphosphate

$\hat{\text{a}}$ -NADH =  $\hat{\text{a}}$ -Nicotinamide Adenine Dinucleotide, Reduced Form

GAPDH = Glyceraldehyde-3-Phosphate Dehydrogenase

G-3-P = Glyceraldehyde 3-Phosphate

$\hat{\text{a}}$ -NAD =  $\hat{\text{a}}$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

P<sub>i</sub> = Inorganic Phosphate

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

**METHOD:** Continuous Spectrophotometric Rate Determination

##### REAGENTS:

- A. 100 mM Triethanolamine 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. **Prepare fresh.**)
- B. 100 mM 3-Phosphoglyceric Acid Solution (3-PGA)  
(Prepare 2 ml in deionized water using D(-)3-Phosphoglyceric Acid, ri(cyclohexylammonium) Salt, Sigma Prod. No. P-8752.)
- C. 200 mM L-Cysteine HCl Solution (Cys)  
(Prepare 1 ml in deionized water using L-Cysteine Hydrochloride, Monohydrate, Sigma Prod. No. C-7880. Neutralize the solution by adding solid Sodium Bicarbonate, Sigma Prod. No. S-8875. **PREPARE FRESH.**)

**Enzymatic Assay of GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE<sup>1</sup>**  
**(EC 1.2.1.12)**

**REAGENTS:** (continued)

- D. 100 mM Magnesium Sulfate Solution (MgSO<sub>4</sub>)  
(Prepare 10 ml in deionized water using Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880.)
- E. 7.0 mM  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form Solution ( $\beta$ -NADH)  
(Prepare 1 ml in deionized water using  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129 or dissolve the contents of one 5 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-105, in the appropriate volume of deionized water. **PREPARE FRESH.**)
- F. 34 mM Adenosine 5'-Triphosphate Solution (ATP)  
(Prepare 1 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394. **PREPARE FRESH.**)
- G. 3-Phosphoglyceric Phosphokinase Enzyme Solution (3-PGK)  
(Immediately before use, prepare a solution containing 200 units/ml in cold deionized water using 3-Phosphoglyceric Phosphokinase, Sigma Prod. No. P-7634.)
- H. Glyceraldehyde-3-Phosphate Dehydrogenase Enzyme Solution (GAPDH)  
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Glyceraldehyde-3-Phosphate Dehydrogenase in cold Reagent A.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.40	2.50
Reagent B (3-PGA)	0.20	0.20
Reagent C (Cys)	0.05	0.05
Reagent D (MgSO <sub>4</sub> )	0.05	0.05
Reagent E ( $\beta$ -NADH)	0.05	0.05
Reagent F (ATP)	0.10	0.10
Reagent G (3-PGK)	0.05	0.05

## Enzymatic Assay of Glyceraldehyde-3-PHOSPHATE DEHYDROGENASE<sup>1</sup> (EC 1.2.1.12)

### PROCEDURE: (continued)

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

	<u>Test</u>	<u>Blank</u>
Reaction H (Enzyme Solution)	0.10	-----

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

### CALCULATIONS:

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

3 = Volume (in milliliters) of assay

df = Dilution factor

0.1 = Volume (in milliliter) of enzyme used

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

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

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

### UNIT DEFINITION:

One unit will reduce 1.0  $\mu$ mole of 3-phosphoglycerate to D-glyceraldehyde-3-phosphate per minute in a coupled system with 3-phosphoglyceric phosphokinase at pH 7.6 at 25°C.

### FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 83 mM triethanolamine, 6.7 mM 3-phosphoglyceric acid, 3 mM L-cysteine, 2 mM magnesium sulfate, 0.1 mM  $\alpha$ -NADH, 1.1 mM ATP, 10 units 3-phosphoglyceric phosphokinase and 0.03 - 0.06 unit glyceraldehyde-3-phosphate dehydrogenase.

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**NOTES:**

1. Not to be used to assay activity of Glyceraldehyde-3-Phosphate Dehydrogenase, from *Bacillus Stearothermophilus*, Sigma Prod. No. G-5892.
2. 3-Phosphoglyceric Phosphokinase unit definition: One unit will convert 1.0 imole of 1,3-diphosphoglycerate to 3-phosphoglycerate per minute at pH 6.9 at 25°C.
3. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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