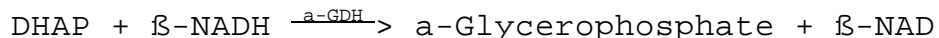
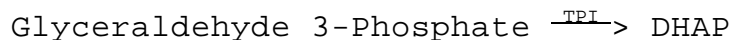
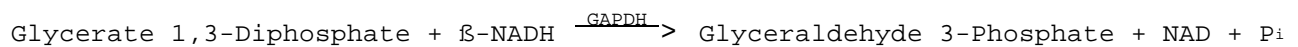
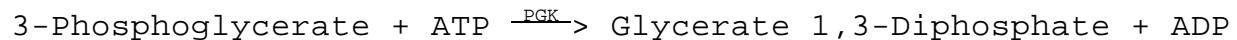
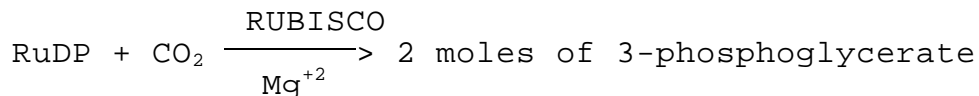


**Enzymatic Assay of D-RIBULOSE 1,5-DIPHOSPHATE CARBOXYLASE
(EC 4.1.1.39)**

PRINCIPLE:



Abbreviations used:

RUDP = D-Ribulose 1,5-Diphosphate

RUBISCO = D-Ribulose 1,5-Diphosphate Carboxylase

ATP = Adenosine 5'-Triphosphate

PGK = 3-Phosphoglyceric Phosphokinase

ADP = Adenosine 5'-Diphosphate

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

GAPDH = Glyceraldehyde 3-Phosphate Dehydrogenase

P_i = Inorganic Phosphate

TPI = Triosephosphate Isomerase

DHAP = Dihydroxyacetone Phosphate

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

a-GDH = a-Glycerophosphate Dehydrogenase

CONDITIONS: T = 25°C, pH = 7.8, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 1 M Tris HCl Buffer, pH 7.8 at 25°C
(Prepare 25 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.8 at 25°C with 10 M HCl.)
- B. 100 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 5 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

**Enzymatic Assay of D-RIBULOSE 1,5-DIPHOSPHATE CARBOXYLASE
(EC 4.1.1.39)**

REAGENTS: (continued)

- C. 2 M Potassium Bicarbonate Solution (KHCO_3)
(Prepare 5 ml in deionized water using Potassium Bicarbonate, Sigma Prod. No. P-9144.)
- D. 2.5 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β -NADH)
(Prepare 5 ml in Reagent A using β -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129 or dissolve the contents of one 1 mg vial of β -Nicotinamide Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. 340-101 in the appropriate volume of Reagent A.)
- E. 100 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 5 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)
- F. 100 mM Glutathione, Reduced Form, Solution (GSH)
(Prepare 5 ml in deionized water using Glutathione, Reduced Form, Free Acid, Sigma Prod. No. G-4251.)
- G. 25 mM D-Ribulose 1,5-Diphosphate Solution (RuDP)
(Prepare 2 ml in deionized water using D-Ribulose 1,5-Diphosphate, Sodium Salt, Hydrate, Sigma Prod. No. R-0878.)
- H. α -Glycerophosphate Dehydrogenase-Triosephosphate Isomerase Enzyme Suspension (α -GDH/TPI)
(Immediately before use prepare a solution containing 50 units/ml of α -Glycerophosphate Dehydrogenase-Triosephosphate Isomerase, Sigma Prod. No. G-1881, in cold deionized water.)
- I. Glyceraldehyde-3-Phosphate Dehydrogenase/3-Phosphoglyceric Phosphokinase Enzyme Solution (GAPDH/3-PGK)
(Immediately before use prepare a solution containing 50 units/ml of Glyceraldehyde-3-Phosphate Dehydrogenase/3-Phosphoglyceric Phosphokinase, Sigma Prod. No. G-8505, in cold deionized water.)
- J. D-Ribulose 1,5-Diphosphate Carboxylase (RUBISCO)
(Immediately before use, prepare a solution containing 0.07 - 0.15 unit/ml of D-Ribulose 1,5-Diphosphate Carboxylase in cold Reagent A.)

**Enzymatic Assay of D-RIBULOSE 1,5-DIPHOSPHATE CARBOXYLASE
(EC 4.1.1.39)**

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Reagent A (Buffer)	4.40
Reagent B (MgCl ₂)	1.50
Reagent C (KHCO ₃)	1.00
Reagent D (β-NADH)	2.40
Reagent E (ATP)	1.50
Reagent F (GSH)	1.50
Reagent G (RuDP)	0.60

Mix by swirling. Adjust to pH 7.8 at 25°C, if necessary, with either 0.1 M NaOH or 0.1 M HCl.

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

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	1.29	1.29
Reagent H (α-GDH/TPI)	0.10	0.10
Reagent I (GAPDH/3-PGK)		0.10
		0.10
Deionized Water	1.41	1.41

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

Reagent J (RUBISCO)	0.10	-----
Reagent A (Buffer)	-----	0.10

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r_{A_{340nm}/\text{min Test}} - r_{A_{340nm}/\text{min Blank}})(3)(df)}{(4)(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

4 = 4 μmoles of β-NADH are oxidized for each μmole of D-Ribulose 1,5-diphosphate utilized

6.22 = Millimolar extinction coefficient for β -NADH at 340
nm
0.1 = Volume (in milliliter) of enzyme used

**Enzymatic Assay of D-RIBULOSE 1,5-DIPHOSPHATE CARBOXYLASE
(EC 4.1.1.39)**

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 D-RuDP and CO₂ to 2.0 μ moles of D-3-phosphoglycerate per minute at pH 7.8 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 259 mM Tris, 5 mM magnesium chloride, 67 mM potassium bicarbonate, 0.2 mM β -nicotinamide adenine dinucleotide, reduced form, 5 mM adenosine 5'-triphosphate, 5 mM glutathione, reduced form, 0.5 mM D-ribulose 1,5-diphosphate, 5 units α -glycerophosphate dehydrogenase-triosephosphate isomerase, 5 units glyceraldehyde-3-phosphate dehydrogenase/3-phosphoglyceric phosphokinase, and 0.007 - 0.015 unit D-ribulose 1,5-diphosphate carboxylase.

REFERENCES:

Racker, E. (1962) *Methods in Enzymology* V, 266-270

Racker, E. (1957) *Archives of Biochemistry and Biophysics* **69**, 300-310

NOTES:

1. 3-Phosphoglyceric Phosphokinase Unit Definition: One unit will convert 1.0 μ mole of 3-phosphoglycerate to 1,3-diphosphoglycerate per minute at pH 7.5 at 25°C.
2. Glyceraldehyde-3-Phosphate Dehydrogenase Unit Definition: One unit will reduce 1.0 μ 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.

**Enzymatic Assay of D-RIBULOSE 1,5-DIPHOSPHATE CARBOXYLASE
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NOTES: (continued)

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 and 25°C.
4. a-Glycerophosphate Dehydrogenase Unit Definition: One unit will convert 1.0 μ mole of dihydroxyacetone phosphate to a-glycerophosphate per minute at pH 7.4 at 25°C.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.