Enzymatic Assay of D-Ribulose 1,5-Diphosphate Carboxylase (EC 4.1.1.39)

**PRINCIPLE:**

\[
\text{RUBISCO} \quad \text{RuDP} + \text{CO}_2 \xrightarrow{\text{Mg}^{2+}} 2 \text{ moles of 3-phosphoglycerate}
\]

\[
3\text{-Phosphoglycerate} + \text{ATP} \xrightarrow{\text{PGK}} \text{Glycerate 1,3-Diphosphate} + \text{ADP}
\]

\[
\text{Glycerate 1,3-Diphosphate} + \beta\text{-NADH} \xrightarrow{\text{GAPDH}} \text{Glyceraldehyde 3-Phosphate} + \text{NAD} + \text{P}_i
\]

\[
\text{Glyceraldehyde 3-Phosphate} \xrightarrow{\text{TPI}} \text{DHAP}
\]

\[
\text{DHAP} + \beta\text{-NADH} \xrightarrow{\text{a-GDH}} \text{a-Glycerophosphate} + \beta\text{-NAD}
\]

**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:**

- \( T = 25^\circ \text{C} \), \( \text{pH} = 7.8 \), \( A_{340\text{nm}} \), 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. Adjust to pH 7.8 at 25°C with 10 M HCl.)

- **B.** 100 mM Magnesium Chloride Solution (MgCl_2)
  (Prepare 5 ml in deionized water using Magnesium Chloride, Hexahydrate.)
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REAGENTS: (continued)

C. 2 M Potassium Bicarbonate Solution (KHCO₃)  
   (Prepare 5 ml in deionized water using Potassium Bicarbonate.)

D. 2.5 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (ß-NADH)  
   (Prepare 5 ml in Reagent A using ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt,  
   or dissolve the contents of one 1 mg vial of ß-Nicotinamide Dinucleotide, Reduced Form, Disodium Salt 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.)

F. 100 mM Glutathione, Reduced Form, Solution (GSH)  
   (Prepare 5 ml in deionized water using Glutathione, Reduced Form, Free Acid.)

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.)

H. a-Glycerophosphate Dehydrogenase-Triosephosphate Isomerase Enzyme Suspension (a-GDH/TPI)  
   (Immediately before use prepare a solution containing 50 units/ml of a-Glycerophosphate Dehydrogenase-Triosephosphate Isomerase, in cold deionized water.)

I. Glycerinaldehyde-3-Phosphate Dehydrogenase/3-Phosphoglyceric Phosphokinase Enzyme Solution (GAPDH/3-PGK)  
   (Immediately before use prepare a solution containing 50 units/ml of Glycerinaldehyde-3-Phosphate Dehydrogenase/3-Phosphoglyceric Phosphokinase, 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.)
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PROCEDURE:

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

- Reagent A (Buffer) 4.40
- Reagent B (MgCl₂) 1.50
- Reagent C (KHCO₃) 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>1.29</td>
<td>1.29</td>
</tr>
<tr>
<td>Reagent H (a-GDH/TPI)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent I (GAPDH/3-PGK)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>1.41</td>
<td>1.41</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the A₃₄₀nm 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 A₃₄₀nm 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

\( r_{A_{340nm}} \) = 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
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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 D-RuDP and CO₂ to 2.0 µmoles 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 a-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. (1957) Archives of Biochemistry and Biophysics 69, 300-310

NOTES:

1. 3-Phosphoglyceric Phosphokinase Unit Definition: One unit will convert 1.0 µmole 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 µmole 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.
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NOTES: (continued)

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 and 25°C.

4. a-Glycerophosphate Dehydrogenase Unit Definition: One unit will convert 1.0 µmole 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 our quality control procedure contact our Technical Service Department.