

**Enzymatic Assay of URIDINE-5'-DIPHOSPHOGLUCOSE
PYROPHOSPHORYLASE
(EC 2.7.7.9)**

PRINCIPLE:

UDPG + PP_i UDPG Pyrophosphorylase > UTP + Glucose 1-Phosphate

Glucose 1-Phosphate Phosphoglucomutase > Glucose 6-Phosphate

Glucose 6-Phosphate + β-NADP G-6-PDH > 6-Phosphogluconate + β-NADPH

Abbreviations used:

UDPG = Uridine 5'-Diphosphoglucose

PP_i = Inorganic Pyrophosphate

UTP = Uridine 5'-Triphosphate

β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate,
Oxidized Form

β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate,
Reduced Form

G-6-PDH = Glucose-6-Phosphate Dehydrogenase

CONDITIONS: T = 25°C, pH = 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Tris HCl Buffer, pH 7.6 at 25°C
(Prepare 50 ml in deionized water using Trizma Base,
Sigma Prod. No. T-1503. Adjust to pH 7.6 at 25°C with
1 M HCl.)
- B. 4.0 mM Uridine 5'-Diphosphoglucose Solution (UDPG)
(Prepare 5 ml in deionized water using Uridine
5'-Diphosphoglucose, Disodium Salt, Sigma
Prod. No. U-4625. **PREPARE FRESH.**)
- C. 300 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 5 ml in deionized water using Magnesium
Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

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REAGENTS: (continued)

- D. 250 mM L-Cysteine Solution (Cys)
(Prepare 5 ml in deionized water using L-Cysteine Hydrochloride, Monohydrate, Sigma Prod. No. C-7880. Adjust to pH 7.0 with solid Sodium Bicarbonate, Sigma Prod. No. S-8875.)
- E. 20 mM β -Nicotinamide Adenine Dinucleotide Phosphate Solution (β -NADP)
(Dissolve the contents of one 30 mg vial of β -Nicotinamide Adenine Dinucleotide Phosphate, Sigma Stock No. 240-310, in the appropriate volume of deionized water **or** prepare 1 ml in deionized water using β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt Sigma Prod. No. N-0505. **PREPARE FRESH.**)
- F. 0.6 mM Glucose 1,6-Diphosphate (G 1,6-P)
(Prepare 1 ml in deionized water using α -D-Glucose 1,6-Diphosphate, Cyclohexylammonium Salt, Hydrate Sigma Prod. No. G-5875.)
- G. Phosphoglucomutase Enzyme Solution (PGLUM)
(Immediately before use, prepare a solution containing 15 units/ml in cold deionized water using Phosphoglucomutase, Sigma Prod. No. P-3397.)
- H. Glucose-6-Phosphate Dehydrogenase (G-6-PDH)
(Immediately before use, prepare a solution containing 15 units/ml in cold deionized water using Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. G-6378.)
- I. 10 mM Tris HCl with 10 mM Magnesium Chloride, pH 7.6 at 25°C (Enzyme Diluent)
(Prepare 25 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503 and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- J. Uridine-5'-Diphosphoglucose Pyrophosphorylase Enzyme Solution
(Immediately before use, prepare a solution containing 0.25 - 0.50 unit/ml of Uridine-5'-Diphosphoglucose Pyrophosphorylase in cold Reagent I.)
- K. 50 mM Sodium Pyrophosphate Solution, pH 7.6 at 25°C (PP_i)
(Prepare 25 ml in deionized water using Tetrasodium Pyrophosphate, Decahydrate Sigma Prod. No. P-9146. Adjust to pH 7.6 at 25°C with 1 M HCl. **PREPARE FRESH.**)

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PROCEDURE:

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

	Test	Blank
Deionized Water	0.27	0.27
Reagent A (Buffer)	1.50	1.50
Reagent B (UDPG)	0.50	0.50
Reagent C (MgCl ₂)	0.16	0.16
Reagent D (Cys)	0.12	0.12
Reagent E (β-NADP)	0.10	0.10
Reagent F (G 1,6-P)	0.05	0.05
Reagent G (PGLUM)	0.05	0.05
Reagent H (G-6-PDH)	0.05	0.05
Reagent J (Enzyme Solution)	0.10	-----
Reagent I (Enzyme Diluent)	-----	0.10

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

Reagent K (PP _i)	0.10	0.10
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Immediately mix by inversion and record the increase 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:

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

3 = Volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β-NADPH at 340 nm

0.1 = Volume (in milliliter) of enzyme used

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

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

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UNIT DEFINITION:

One unit will cause the formation of 1.0 μ mole of glucose 1-phosphate from uridine 5'-diphosphoglucose and inorganic pyrophosphate per minute at pH 7.6 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 50 mM Tris, 0.67 mM uridine 5'-diphosphoglucose, 16 mM magnesium chloride, 10 mM L-cysteine, 0.67 mM β -nicotinamide adenine dinucleotide phosphate, 0.01 mM glucose 1,6-diphosphate, 0.75 unit phosphoglucomutase, 0.75 unit glucose 6-phosphate dehydrogenase, 1.7 mM sodium pyrophosphate and 0.025 - 0.05 unit uridine-5'-diphosphoglucose pyrophosphorylase.

REFERENCE:

Bergmeyer, H.U., Gawehn, K. and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed) 2nd ed., Volume I, pp 519-520, Academic Press, Inc. New York

NOTES:

1. Glucose-6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0 μ mole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of β -NADP at pH 7.4 at 25°C.
2. Phosphoglucomutase Unit Definition: One unit will convert 1.0 μ mole of α -D-glucose 1-phosphate to α -D-glucose 6-phosphate per minute at pH 7.4 at 30°C.
3. This assay is based on the cited reference.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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