



SIGMA QUALITY CONTROL TEST PROCEDURE

Product Information

Enzymatic Assay of URIDINE-5'-DIPHOSPHOGLUCOSE PYROPHOSPHORYLASE (EC 2.7.7.9) Sigma Prod. No. U-8501

PRINCIPLE:

UDPG + PP_i $\xrightarrow{\text{UDPG Pyrophosphorylase}}$ UTP + Glucose 1-Phosphate

Glucose 1-Phosphate $\xrightarrow{\text{Phosphoglucomutase}}$ Glucose 6-Phosphate

Glucose 6-Phosphate + β -NADP $\xrightarrow{\text{G-6-PDH}}$ 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.)

Enzymatic Assay of URIDINE-5'-DIPHOSPHOGLUCOSE PYROPHOSPHORYLASE
(EC 2.7.7.9)
Sigma Prod. No. U-8501

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.**)

**Enzymatic Assay of URIDINE-5'-DIPHOSPHOGLUCOSE PYROPHOSPHORYLASE
(EC 2.7.7.9)
Sigma Prod. No. U-8501**

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
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
------------------------------	------	------

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

CALCULATIONS:

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

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

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

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

**Enzymatic Assay of URIDINE-5'-DIPHOSPHOGLUCOSE PYROPHOSPHORYLASE
(EC 2.7.7.9)
Sigma Prod. No. U-8501**

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.

Sigma warrants that the above procedure information is currently utilized at Sigma and that Sigma products conform to the information in Sigma publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of Sigma products, see reverse side of invoice or packing slip for additional terms and conditions of sale.