

**Enzymatic Assay of URIDINE 5'-DIPHOSPHOGALACTOSE 4-EPIMERASE
(EC 5.1.3.2)**

PRINCIPLE:

UDP-Gal UDP-Gal 4-Epimerase > UDPG

UDPG + 2 β -NAD + H₂O UDPG Dehydrogenase > UDP-Glucuronate + 2 β -NADH

Abbreviations used:

UDP-Gal = Uridine 5'-Diphosphogalactose

UDPG = Uridine 5'-Diphosphoglucose

UDP-Gal 4-Epimerase = Uridine 5'-Diphosphogalactose
4-Epimerase

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

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

UDP-Glucuronate = Uridine 5'-Diphosphoglucuronate

CONDITIONS: T = 25°C, pH = 8.8, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 1000 mM Glycine Buffer, pH 8.8 at 25°C
(Prepare 100 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126. Adjust to pH 8.8 at 25°C with 1 M NaOH.)
- B. 5 mM Uridine 5'-Diphosphogalactose Solution (UDP-Gal)
(Prepare 5 ml in deionized water using Uridine 5'-Diphosphogalactose, Sodium Salt, Sigma Prod. No. U-4500.)
- C. 50 mM β -Nicotinamide Adenine Dinucleotide Solution (β -NAD)
(Prepare 2 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004 or dissolve the contents of one 50 mg vial of β -Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-150, in the appropriate volume of deionized water.
PREPARE FRESH.)

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REAGENTS: (continued)

- D. Uridine 5'-Diphosphoglucose Dehydrogenase Enzyme Solution (UDPGDH)
(Immediately before use, prepare a solution containing 2 units/ml in cold deionized water using Uridine 5'-Diphosphoglucose Dehydrogenase, Sigma Prod. No. U-5500.)
- E. 100 mM Citric Acid Solution, pH 7.0 at 25°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate, Sigma Prod. No. C-7129. Adjust to pH 7.0 at 25°C with 1 M NaOH.)
- F. Uridine 5'-Diphosphogalactose 4-Epimerase Enzyme Solution
(Immediately before use, prepare a solution containing 0.06 - 0.13 unit/ml of Uridine 5'-Diphosphogalactose 4-Epimerase in cold Reagent E.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Deionized Water	2.38	2.38
Reagent A (Buffer)	0.30	0.30
Reagent B (UDP-Gal)	0.06	0.06
Reagent C (β-NAD)	0.06	0.06
Reagent D (UDPGDH)	0.10	0.10

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

Reagent E (Enzyme Diluent)	-----	0.10
Reagent F (Enzyme Solution)	0.10	-----

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.

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CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r_{A_{340\text{nm}}/\text{min Test}} - r_{A_{340\text{nm}}/\text{min Blank}})(3.0)(\text{df})}{(2)(6.22)(0.10)}$$

3.0 = Total volume (in milliliters) of assay

df = Dilution factor

2 = 2 Moles of β -NADH produced per mole UDPG oxidized

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

0.10 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will convert 1.0 μ mole of UDP-galactose to UDP-glucose per minute at pH 8.8 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 100 mM glycine, 0.1 mM uridine 5'-diphosphogalactose, 1 mM β -NAD, 0.2 unit uridine-5'-diphosphoglucose dehydrogenase, 3.3 mM citric acid and 0.006 - 0.013 unit uridine 5'-diphosphogalactose 4-epimerase.

REFERENCE:

Fukasawa, T. et al. (1980) *Journal of Biological Science* **255**, 2705-2707.

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.
3. Uridine 5'-Diphosphoglucose Dehydrogenase Unit
Definition: One unit will oxidize 1.0 μ mole of UDP-

glucose to UDP-glucuronic acid per min at pH 8.7 at 25°C.

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