

**Enzymatic Assay of URIDINE-5'-DIPHOSPHOGLUCOSE DEHYDROGENASE
(EC 1.1.1.22)**

PRINCIPLE:

UDPG + 2 β -NAD $\xrightarrow{\text{UDPG Dehydrogenase}}$ UDP-Glucuronate + 2 β -NADH

Abbreviations used:

UDPG = Uridine 5'-Diphosphoglucose

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

UDP-Glucuronate = Uridine 5'-Diphosphoglucuronate

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

CONDITIONS: T = 25°C, pH = 8.7, $A_{340\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Glycine Buffer, pH 8.7 at 25°C
(Prepare 100 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126. Adjust to pH 8.7 at 25°C with 1 M NaOH.)
- B. 3 mM Uridine 5'-Diphosphoglucose Solution (UDPG)
(Prepare 5 ml in Reagent A using Uridine 5'-Diphosphoglucose, Disodium Salt, Sigma Prod. No. U-4625.)
- C. 30 mM β -Nicotinamide Adenine Dinucleotide Solution (β -NAD)
(Dissolve the contents of one 20 mg vial of β -Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-120, in the appropriate volume of deionized water or use β -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004. **PREPARE FRESH.**)
- D. Uridine-5'-Diphosphoglucose Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Uridine-5'-Diphosphoglucose Dehydrogenase in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.60	2.60
Reagent B (UDPG)	0.20	0.20
Reagent C (β -NAD)	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:

Deionized Water	-----	0.10
Reagent D (Enzyme Solution)	0.10	-----

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3)(df)}{(2)(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

2 = 2 Moles of β -NADH formed per mole of Uridine
5'-Diphosphoglucose oxidized

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

0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will oxidize 1.0 μmole of UDP-glucose to

UDP-glucuronic acid per minute at pH 8.7 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 93 mM glycine, 0.2 mM uridine 5'-diphosphoglucose, 1 mM β -nicotinamide adenine dinucleotide, and 0.01 - 0.02 unit uridine-5'-diphosphoglucose dehydrogenase.

REFERENCE:

Maxwell, E.S, Kalckar, H.M. and Strominger, J.L. (1956)
Archives of Biochemistry and Biophysics **65**, 2-10

NOTES:

1. This assay is based on the cited reference.
2. 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.