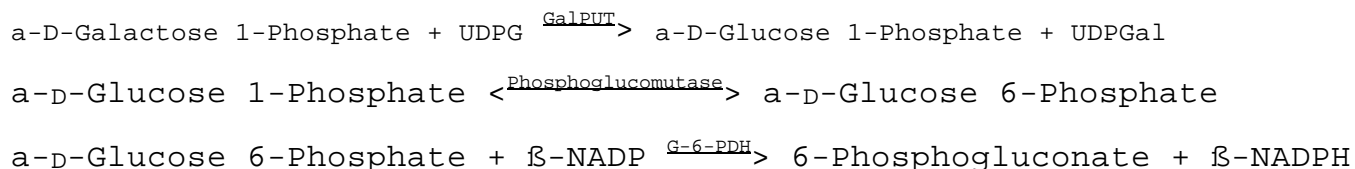


**Enzymatic Assay of GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE
(EC 2.7.7.12)**

PRINCIPLE:



Abbreviations used:

UDPG = Uridine 5'-Diphosphoglucose

GalPUT = Galactose-1-Phosphate Uridyl Transferase

UDPGal = Uridine 5'-Diphosphogalactose

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

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

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

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

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 1000 mM Glycine Buffer, pH 8.7 at 25°C
(Prepare 25 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. 40 mM Galactose 1-Phosphate Solution (Gal 1-P)
(Prepare 2 ml in deionized water using a-D-Galactose 1-Phosphate, Dipotassium Salt, Sigma Prod. No. G-0380.)
- C. 10 mM Uridine 5'-Diphosphoglucose Solution (UDPG)
(Prepare 3 ml in deionized water using Uridine 5'-Diphosphoglucose, Disodium Salt, Sigma Prod. No. U-4625.)

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REAGENTS: (continued)

- D. 0.2 mM Glucose 1,6-Diphosphate Solution (G 1,6-DiP)
(Prepare 1 ml in deionized water using α -D-Glucose 1,6-Diphosphate, Cyclohexylammonium Salt, Hydrate, Sigma Prod. No. G-5875.)
- E. 20 mM β -Nicotinamide Adenine Dinucleotide Phosphate Solution (β -NADP)
(Prepare 2 ml in deionized water using β -Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505. **PREPARE FRESH.**)
- F. 300 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 5 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- G. 200 mM L-Cysteine Hydrochloride Solution (Cys)
(Prepare 2 ml in deionized water using L-Cysteine Hydrochloride, Monohydrate, Sigma Prod. No. C-7880. Neutralize by adding solid Sodium Bicarbonate, Sigma Prod. No. S-8875.)
- H. Phosphoglucomutase Solution (PGLUM)
(Immediately before use, prepare a solution containing 15 units/ml in cold deionized water using Phosphoglucomutase, Sigma Prod. No. P-3397.)
- I. Glucose-6-Phosphate Dehydrogenase Solution (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.)
- J. 100 mM Citrate Solution, pH 7.5 at 25°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Citric Acid, Trisodium Salt, Dihydrate, Sigma Prod. No. C-7254. Adjust to pH 7.5 at 25°C with 1 M HCl.)
- K. Galactose-1-Phosphate Uridyl Transferase Enzyme Solution (GalPUT)
(Immediately before use, prepare a solution containing 0.2 - 0.8 unit/ml of Galactose-1-Phosphate Uridyl Transferase in cold Reagent J.)

**Enzymatic Assay of GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE
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PROCEDURE:

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

| | |
|----------------------------|-------|
| Deionized water | 17.50 |
| Reagent A (Buffer) | 3.00 |
| Reagent B (Gal 1-P) | 1.00 |
| Reagent C (UDPG) | 2.00 |
| Reagent D (G 1,6-DP) | 1.00 |
| Reagent E (β -NADP) | 1.00 |
| Reagent F ($MgCl_2$) | 1.00 |
| Reagent G (Cys) | 1.50 |

Mix and adjust to pH 8.7 at 25°C with 100 mM HCl or 100 mM NaOH, if necessary.

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

| | <u>Test</u> | <u>Blank</u> |
|---------------------|-------------|--------------|
| Reaction Cocktail | 2.80 | |
| | | 2.80 |
| Reagent H (PGLUM) | 0.05 | 0.05 |
| Reagent I (G-6-PDH) | 0.05 | 0.05 |

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

| | | |
|----------------------------|-------|------|
| Reagent J (Enzyme Diluent) | ----- | 0.10 |
| Reagent K (GalPUT) | 0.10 | --- |
| | | --- |

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

CALCULATIONS:

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

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

RM = Reaction Mix

**Enzymatic Assay of GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE
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UNIT DEFINITION:

One unit will form 1.0 μ mole of glucose 1-phosphate from UDP-glucose, galactose 1-phosphate and NADP⁺ per minute at pH 8.7 at 25°C as detected by a coupled system using phosphoglucomutase and β -NADP.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 100 mM glycine, 1.3 mM galactose 1-phosphate, 0.67 mM uridine 5'-diphosphoglucose, 0.0067 mM glucose-1,6-diphosphate, 0.67 mM β -NADP, 10 mM MgCl₂, 10 mM L-cysteine hydrochloride, 3.3 mM citrate, 0.75 unit phosphoglucomutase, 0.75 unit glucose-6-phosphate dehydrogenase and 0.02 - 0.08 unit galactose-1-phosphate uridyl transferase.

REFERENCES:

Mayes, J.S. and Hanson R.G. (1966) *Methods in Enzymology* IX, 708-713.

NOTES:

1. This assay is a modification of the procedure described in the cited reference.
2. 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.
3. 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.
4. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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