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

PRINCIPLE:

\[
\text{a-D-Galactose 1-Phosphate} + \text{UDPG} \xrightarrow{\text{GalPUT}} \text{a-D-Glucose 1-Phosphate} + \text{UDPGal}
\]

\[
\text{a-D-Glucose 1-Phosphate} \xrightarrow{\text{Phosphoglucomutase}} \text{a-D-Glucose 6-Phosphate}
\]

\[
\text{a-D-Glucose 6-Phosphate} + \beta-\text{NADP} \xrightarrow{\text{G-6-PDH}} \text{6-Phosphogluconate} + \beta-\text{NADPH}
\]

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\text{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. 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.)

C. 10 mM Uridine 5'-Diphosphoglucose Solution (UDPG)  
(Prepare 3 ml in deionized water using Uridine 5'-Diphosphogluucose, Disodium Salt.)
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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.)

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. PREPARE FRESH.)

F. 300 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 5 ml in deionized water using Magnesium Chloride, Hexahydrate.)

G. 200 mM L-Cysteine Hydrochloride Solution (Cys)
(Prepare 2 ml in deionized water using L-Cysteine Hydrochloride, Monohydrate. Neutralize by adding solid Sodium Bicarbonate.)

H. Phosphoglucomutase Solution (PGLUM)
(Immediately before use, prepare a solution containing 15 units/ml in cold deionized water using Phosphoglucomutase.)

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.)

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. 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.)
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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 (MgCl₂) 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>2.80</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent H (PGLUM)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent I (G-6-PDH)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the $A_{340\text{nm}}$ 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 $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r_{A_{340\text{nm}}/\text{minute}}$ using the maximum linear rate for both the Test and Blank.

CALculATIONS:

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

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

RM = Reaction Mix
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UNIT DEFINITION:

One unit will form 1.0 µmole 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 uridylyl transferase.

REFERENCES:


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 µmole 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 µmole 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 OUR product and stock numbers.

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