Enzymatic Assay of GALACTOSYLTRANSFERASE
(EC 2.4.1.22)

PRINCIPLE:

\[ \text{UDP-Galactose} + \text{D-Glucose} \xrightarrow{\text{Galactosyltransferase}} \text{UDP} + \text{Lactose} \]
\[ \text{UDP} + \text{PEP} \xrightarrow{\text{PK}} \text{Pyruvate} + \text{UTP} \]
\[ \text{Pyruvate} + \text{β-NADH} \xrightarrow{\text{LDH}} \text{Lactate} + \text{β-NAD} \]

Abbreviations used:
- UDP-Galactose = Uridine 5'-Diphosphogalactose
- UDP = Uridine 5'-Diphosphate
- PEP = Phospho(enol)pyruvate
- PK = Pyruvate Kinase
- β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
- LDH = Lactic Dehydrogenase
- β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS: \( T = 30^\circ C, \text{pH} = 8.4, A_{340nm}, \text{Light path} = 1 \text{ cm} \)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 250 mM Glycylglycine Buffer, pH 8.6 at 30°C.
   (Prepare 50 ml in deionized water using Gly-Gly, Hydrochloride, Sigma Prod. No. G-1127. Adjust to pH 8.6 at 30°C with 1 M NaOH.)

B. 0.70 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form (β-NADH)
   (Dissolve the contents of one 5 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-105, in the appropriate volume of deionized water. PREPARE FRESH.)

C. 6.4 mM Phospho(enol)pyruvate Solution (PEP)
   (Prepare 10 ml in deionized water using Phospho(enol)Pyruvate Tri(Cyclohexylammonium) Salt, Sigma Prod. No. P-7252.)
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REAGENTS: (continued)

D. 100 mM Manganese Chloride Tetrahydrate with 1 M Potassium Chloride Solution (MnCl₂/KCl)  
(Prepare 5.0 ml in deionized water using Manganese Chloride Tetrahydrate, Sigma Prod. No. M-3634 and Potassium Chloride, Sigma Prod. No. P-4504.)

E. 5.65 mM Uridine 5'-Diphosphogalactose Solution (UDP-Galactose)  
(Prepare 10 ml in deionized water using Uridine 5'-Diphosphogalactose, Sodium Salt, Sigma Prod. No. U-4500.)

F. 50 mM Glycylglycine Buffer, pH 8.0 at 30°C.  
(Prepare 10 ml in deionized water using Gly-Gly, Hydrochloride, Sigma Prod. No. G-1127. Adjust to pH 8.0 at 30°C with 1 M NaOH.)

G. 0.6% (w/v) a-Lactalbumin Solution  
(Prepare 2 ml in Reagent F using a-Lactalbumin, Sigma Prod. No. L-6010.)

H. 286 mM D-Glucose Solution  
(Prepare 10 ml in deionized water using β-D(+)-Glucose, Sigma Prod. No. G-5250.)

I. PK/LDH Enzymes Suspension¹  
(Use PK/LDH Enzymes Suspension, Sigma Stock No. 40-7.)

J. 20 mM Tris HCl Buffer with 2 mM Ethylenediaminetetraacetic Acid and 2 mM 2-Mercaptoethanol, pH 7.5 at 30°C (Enz Dil)  

K. Galactosyltransferase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Galactosyltransferase in cold Reagent J.)
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PROCEDURE:  

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

| Reagent A (Buffer) | 5.00 |
| Reagent B (β-NADH) | 5.00 |
| Reagent C (PEP)    | 5.00 |
| Reagent D (MnCl₂/KCl) | 1.25 |

Mix and adjust to pH 8.4 at 30°C with 1 M HCl or 1 M NaOH, if necessary.  

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>0.50</td>
</tr>
<tr>
<td>Reaction Cocktail</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent E (UDP-Galactose)</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent G (α-Lactalbumin)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent I (PK/LDH Suspension)</td>
<td>0.025</td>
</tr>
<tr>
<td>Reagent J (Enz Dil)</td>
<td>------</td>
</tr>
<tr>
<td>Reagent K (Enzyme Solution)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 30°C. Monitor the A₃₄₀ nm until constant, using a suitably thermostatted spectrophotometer. Then add:  

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent H (D-Glucose)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in A₃₄₀ nm for approximately 10 minutes. Obtain the r A₃₄₀ nm/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:  

Units/ml enzyme = \[
\frac{(A_{340\text{nm/min Test}} - A_{340\text{nm/min Blank}}) (3.065) (df)}{(6.22)(0.04)}
\]

3.065 = Total volume (in milliliters) of assay  
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm

SPUDPG01  
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0.04 = Volume (in milliliter) of enzyme used
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CALCULATIONS: (continued)

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

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

UNIT DEFINITION:

One unit will transfer 1.0 µmole of galactose from UDP-galactose to D-glucose per minute at pH 8.4 at 30°C in the presence of 0.2 mg of α-lactalbumin per ml of reaction mixture.

FINAL ASSAY CONCENTRATIONS:

In a 3.065 ml reaction mix, the final concentrations are 52 mM glycylglycine, 0.14 mM β-nicotinamide adenine dinucleotide, 1.3 mM phospho(enol)pyruvate, 5.0 mM manganese chloride, 50 mM potassium chloride, 0.37 mM uridine 5'-diphosphogalactose, 0.02% (w/v) α-lactalbumin, 19 mM glucose, 0.26 mM Tris, 0.03 mM ethylenediaminetetraacetic acid, 0.03 mM 2-mercaptoethanol, 17.5 units pyruvate kinase, 25 units lactic dehydrogenase and 0.004 - 0.008 unit galactosyltransferase.

REFERENCES:


NOTES:

1. Contains not less than 700 Pyruvate Kinase units and 1000 Lactic Dehydrogenase units per ml.

2. Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 µmole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
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NOTES: (continued)

3. Pyruvate Kinase Unit Definition: One unit will convert 1.0 µmole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.

4. a-Lactalbumin is included in the assay since, according to Fitzgerald et al., it lowers the apparent Km of glucose.

5. This assay is based on the cited references.

6. 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.