

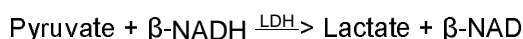
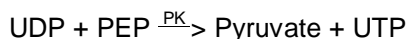


Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of GALACTOSYLTRANSFERASE (EC 2.4.1.22)

PRINCIPLE:



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°C, pH = 8.4, $A_{340\text{nm}}$, Light path = 1 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.)

**Enzymatic Assay of GALACTOSYLTRANSFERASE
(EC 2.4.1.22)**

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) α-Lactalbumin Solution
(Prepare 2 ml in Reagent F using α-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)
(Prepare 50 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253, Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Sigma Stock No. ED4SS and 2-Mercaptoethanol, Sigma Prod. No. M-6250. Adjust to pH 7.5 at 30°C with 1 M NaOH.)
- K. Galactosyltransferase Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Galactosyltransferase in cold Reagent J.)

**Enzymatic Assay of GALACTOSYLTRANSFERASE
(EC 2.4.1.22)**

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 ($MnCl_2/KCl$)	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:

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

Mix by inversion and equilibrate to 30°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent H (D-Glucose)	0.20	0.20
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Immediately mix by inversion and record the decrease in A_{340nm} for approximately 10 minutes. Obtain the $\Delta A_{340nm}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3.065 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.04 = Volume (in milliliter) of enzyme used

**Enzymatic Assay of GALACTOSYLTRANSFERASE
(EC 2.4.1.22)**

CALCULATIONS: (continued)

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

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

UNIT DEFINITION:

One unit will 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 ethylenediamine-tetraacetic acid, 0.03 mM 2-mercaptoethanol, 17.5 units pyruvate kinase, 25 units lactic dehydrogenase and 0.004 - 0.008 unit galactosyltransferase.

REFERENCES:

Brodbeck, U. and Ebner, K.E. (1966) *Journal of Biological Chemistry* **241**, 762-764

Fitzgerald, D.K., Brodbeck, U., Kiyosawa, I., Mawal, R., Colvin, B., and Ebner, K.E., (1970) *Journal of Biological Chemistry* **245**, 2103-2108

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.

**Enzymatic Assay of GALACTOSYLTRANSFERASE
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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. α -Lactalbumin is included in the assay since, according to Fitzgerald et al., it lowers the apparent K_m 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.

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