SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of GALACTOSYLTRANSFERASE
(EC 2.4.1.22)

PRINCIPLE:

UDP-Galactose + D-Glucose $\xrightarrow{\text{Galactosyltransferase}}$ UDP + Lactose

UDP + PEP $\xrightarrow{\text{PK}}$ Pyruvate + UTP

Pyruvate + β-NADH $\xrightarrow{\text{LDH}}$ Lactate + 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°C, pH = 8.4, A_{340nm}, 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.)

REAGENTS: (continued)

D. 100 mM Manganese Chloride Tetrahydrate with 1 M Potassium Chloride Solution ($MnCl_2$/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 Suspension[1]
(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.)

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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 ($MnCl_2$/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:

	Test	Blank
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

Immediately mix by inversion and record the decrease in A_{340nm} for approximately 10 minutes. Obtain the ΔA_{340nm}/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

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.

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 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.