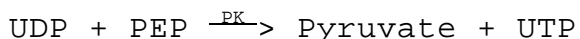
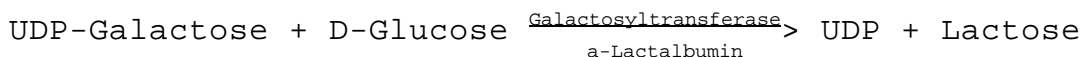


## Suitability Assay for $\alpha$ -LACTALBUMIN

### PRINCIPLE:



Abbreviations used:

UDP-Galactose = Uridine 5'-Diphosphogalactose

UDP = Uridine 5'-Diphosphate

PEP = Phospho(enol)pyruvate

UTP = Uridine 5'-Triphosphate

PK = Pyruvate Kinase

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

LDH = Lactic Dehydrogenase

$\beta$ -NAD =  $\beta$ -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. 0.32 mM  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form ( $\beta$ -NADH)  
(Dissolve the contents of one 5 mg vial of  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Stock No. 340-105, in the appropriate volume of deionized water. **PREPARE FRESH.**)
- C. Phospho(enol)pyruvate (PEP)  
(Use Phospho(enol)Pyruvate Tri(cyclohexylammonium) Salt, Sigma Prod. No. P-7252.)

## Suitability Assay for $\alpha$ -LACTALBUMIN

### REAGENTS: (continued)

- D. 100 mM Manganese Chloride Tetrahydrate with 1 M Potassium Chloride Solution ( $\text{MnCl}_2/\text{KCl}$ )  
(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. 4.8 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. 0.3% (w/v)  $\alpha$ -Lactalbumin Solution  
(Prepare 2 ml in Reagent F using  $\alpha$ -Lactalbumin, Sigma Prod. No. L-6010.)
- H. 1.0 M D-Glucose Solution  
(Prepare 2 ml in deionized water using  $\beta$ -D(+)Glucose, Sigma Prod. No. G-5250.)
- I. PK/LDH Enzymes Suspension<sup>1</sup>  
(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 0.05 - 0.15 unit/ml of Galactosyltransferase, Sigma Prod. No. G-5507, in cold Reagent J.)

## Suitability Assay for $\alpha$ -LACTALBUMIN

### PROCEDURE:

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

Reagent A (Buffer)	10.00
Reagent B ( $\beta$ -NADH)	20.00
Reagent D ( $\text{MnCl}_2/\text{KCl}$ )	2.50
Reagent C (PEP)	30 mg

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	0.25	0.25
Reaction Cocktail	2.00	2.00
Reagent E (UDP-Galactose)	0.20	0.20
Reagent G ( $\alpha$ -Lactalbumin)	0.20	0.20
Reagent I (PK/LDH Suspension)	0.05	0.05
Reagent H (D-Glucose)	0.20	0.20

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

Reagent K (Galactosyltransferase)	0.10	-----
Reagent J (Enz Dil)	-----	0.10

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

### CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{340\text{nm}}/\text{min Test} - A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(6.22)(0.01)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -NADH at 340 nm

0.1 = Volume (in milliliter) of enzyme used

## Suitability Assay for $\alpha$ -LACTALBUMIN

### 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  $\mu$ mole 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  $\alpha$ -lactalbumin per ml of reaction mixture.

### SPECIFICATION:

The activity of Galactosyltransferase, Sigma Prod. No. G-5507, is compared to that of a control.

### FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 55 mM glycylglycine, 0.13 mM  $\beta$ -nicotinamide adenine dinucleotide, reduced form, 1.3 mM phospho(enol)pyruvate, 5.1 mM manganese chloride, 51.2 mM potassium chloride, 0.32 mM uridine 5'-diphosphogalactose, 0.02% (w/v)  $\alpha$ -lactalbumin, 67 mM glucose, 0.67 mM Tris, 0.07 mM ethylenediaminetetraacetic acid, 0.07 mM 2-mercaptoethanol, 35 units pyruvate kinase, 50 units lactic dehydrogenase, and 0.005 - 0.015 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.

## Suitability Assay for $\alpha$ -LACTALBUMIN

### NOTES: (continued)

2. L-Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0  $\mu$ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
3. Pyruvate Kinase Unit Definition: One unit will convert 1.0  $\mu$ mole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.
4.  $\alpha$ -Lactalbumin is included in the assay since, according to Fitzgerald D.K. et al., it lowers the apparent  $K_m$  of glucose.
5. Galactosyltransferase Unit Definition: One unit will transfer 1.0  $\mu$ mole of galactose from UDP-galactose to D-glucose per minute at pH 8.4 at 30°C in the presence of 0.2 mg  $\alpha$ -lactalbumin per ml of reaction mixture.
6. This assay is based on the cited references.
7. 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.**