**Enzymatic Assay of GALACTOKINASE**
**(EC 2.7.1.6)**

**PRINCIPLE:**

$_{D}$(+)-Galactose + ATP $\xrightarrow{GK}$ a-$_{D}$-Galactose-1-Phosphate + ADP

ADP + PEP $\xrightarrow[Mg^{++},\ K^{+}]{PK}$ Pyruvate + ATP

Pyruvate + ß-NADH $\xrightarrow{LDH}$ Lactate + ß-NAD

Abbreviations:
ATP = Adenosine 5'-Triphosphate
ADP = Adenosine 5'-Diphosphate
PEP = Phospho(enol)pyruvate
PK = Pyruvate Kinase
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
LDH = Lactic Dehydrogenase

**CONDITIONS:** T = 25°C, pH = 7.0, $A_{340nm}$, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 160 mM Potassium Phosphate Buffer, pH 7.0 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Prod. No. P-5379. Adjust to pH 7.0 at 25°C with 1 M NaOH.)

B. 100 mM $_{D}$-Galactose Substrate Solution
(Prepare 1.0 ml in deionized water using $_{D}$(+)-Galactose, Prod. No. G-0750.)

C. 5.9 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 5.0 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Prod. No. A-5394.)

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**REAGENTS:** (continued)

D. 16.2 mM Phospho(enol)pyruvate (PEP)
(Prepare 5 ml in deionized water using Phospho(enol)pyruvate, Trisodium Salt, Prod. No. P-7002.)

E. 800 mM Potassium Chloride Solution (KCL)
(Prepare 5 ml in deionized water using Potassium Chloride, Prod. No. P-4504.)

F. 100 mM Magnesium Chloride ($MgCl_2$)
(Prepare 5 ml in deionized water using Magnesium Chloride, Hexahydrate, Prod. No. M-0250.)

G. 20 mM Ethylenediaminetetraacetic Acid (EDTA)
(Prepare 5 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Stock No. ED4S.)

H. 3.76 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form, (ß-NADH)
(Dissolve the contents of one 5 mg vial of ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt,
Stock No. 340-105, in 1.70 ml of Reagent A.)

I. PK/LDH Mixed Enzyme Solution (PK/LDH)[1]
(Use PK/LDH Enzyme Suspension, Stock No. 40-7.)

J. Galactokinase Enzyme Solution (GK)
(Immediately before use, prepare a solution containing 0.25 - 0.75 units/ml of Galactokinase in cold, deionized water.)

**PROCEDURE:**

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

| | |
|---|---|
| Reagent A (Buffer) | 11.5 |
| Reagent C (ATP) | 3.0 |
| Reagent D (PEP) | 3.0 |
| Reagent E (KCL) | 3.0 |
| Reagent F ($MgCl_2$) | 3.0 |
| Reagent G (EDTA) | 3.0 |
| Reagent H (ß-NADH) | 1.0 |

Mix and adjust to pH 7.0 at 25°C with 1 M HCl or 1 M NaOH,

if necessary.

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**PROCEDURE:** (continued)

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

| | Test | Blank |
|---|---|---|
| Reaction Mix Cocktail | 2.75 | 2.75 |
| Reagent I (PK/LDH) | 0.05 | 0.05 |

Mix by inversion and equilibrate to 25°C. Monitor the $A_{340nm}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

| | | |
|---|---|---|
| Reagent J (GK) | 0.10 | ------ |
| Deionized Water | ------ | 0.10 |

Mix by inversion and monitor the $A_{340nm}$ until constant. Then add:

| | | |
|---|---|---|
| Reagent B ($_D$-Galactose) | 0.10 | 0.10 |

Immediately mix by inversion and record the decrease in $A_{340nm}$ for approximately 5 minutes. Obtain the $\Delta A_{340nm}$/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

6.22 = Millimolar extinction coefficient of ß-NADH at 340nm
RM = Reaction Mix

**UNIT DEFINITION:**

One unit will convert 1.0 µmole of $_D$-galactose to galactose 1-phosphate per minute at pH 7.0 at 25°C.

**FINAL ASSAY CONCENTRATION:**

In a 3 ml reaction mix, the final concentrations are 67 mM potassium phosphate, 0.6 mM ATP, 1.6 mM PEP, 80 mM KCL, 10 mM $MgCl_2$, 2 mM EDTA, 0.13 mM ß-NADH, 3.3 mM $_D$(+)-galactose, 35 units pyruvate kinase, 50 units lactic dehydrogenase and .025 - 0.075 units galactokinase.

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**REFERENCES:**

(1966) Methods in Enzymology, **IX**, 407

**NOTES:**

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

2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.