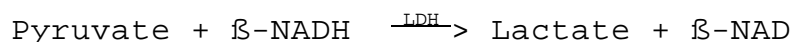
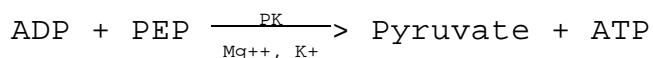
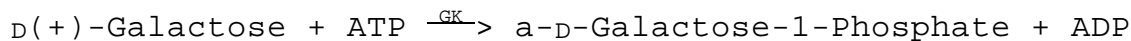


**Enzymatic Assay of GALACTOKINASE
(EC 2.7.1.6)**

PRINCIPLE:



Abbreviations:

ATP = Adenosine 5'-Triphosphate

ADP = Adenosine 5'-Diphosphate

PEP = Phospho(enol)pyruvate

PK = Pyruvate Kinase

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

β -NAD = β -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 (MgCl₂)
(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 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, 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 (β-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:

	<u>Test</u>	<u>Blank</u>
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 $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

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

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

Reagent B (D-Galactose)	0.10	0.10
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Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{340\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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

RM = Reaction Mix

UNIT DEFINITION:

One unit will convert 1.0 μ mole 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 β -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.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.