

**Enzymatic Assay of CHOLINE KINASE
(EC 2.7.1.32)**

PRINCIPLE:

Choline + ATP $\xrightarrow{\text{CK}}$ o-Phosphocholine + ADP

ADP + PEP $\xrightarrow{\text{PK}}$ ATP + Pyruvate

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

Abbreviations used:

ATP = Adenosine 5'-Triphosphate

CK = Choline Kinase

ADP = Adenosine 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 = 25°C, pH = 8.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Glycylglycine Buffer with 60 mM Potassium Chloride, 10 mM Magnesium Chloride, 4 mM Ethylenediaminetetraacetic Acid, and 0.2 mM β -Nicotinamide Adenine Dinucleotide, Reduced Form, pH 8.5 at 25°C (React Cocktail)
(Prepare 30 ml in deionized water using Gly-Gly, Free Base, Sigma Prod. No. G-1002, Potassium Chloride, Sigma Prod. No. P-4504, Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250, Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4SS, and β -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. Adjust to pH 8.5 at 25°C with either 1 M HCl or 1 M NaOH.)
- B. 49 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 1 ml in deionized water using Phospho(enol)pyruvate, Monopotassium Salt, Sigma Prod. No. P-7127.)

**Enzymatic Assay of CHOLINE KINASE
(EC 2.7.1.32)**

REAGENTS: (continued)

- C. 30 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 1 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)
- D. 100 mM Choline Chloride Solution (CC)
(Prepare 1 ml in deionized water using Choline Chloride Salt, Sigma Prod. No. C-1879.)
- E. PK/LDH Enzymes Solution¹ (PK/LDH)
(Use PK/LDH Enzymes, Sigma Prod. No. P-0294.)
- F. 10 mM Tris Acetate Buffer with 13 mM β -Mercaptoethanol,
1 mM Ethylenediaminetetraacetic Acid, and 15 mM Magnesium Chloride, pH 7.2 at 25°C (Enz Dil)
(Prepare 25 ml in deionized water using Trizma Acetate, Sigma Prod. No. T-1258, 2-Mercaptoethanol, Sigma Prod. No. M-6250, Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4SS, and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250. Adjust to pH 7.2 at 25°C with either 1 M HCl or 1 M NaOH.)
- G. Choline Kinase Enzyme Solution (CK)
(Immediately before use, prepare a solution containing 0.25 - 0.50 unit/ml of Choline Kinase in cold Reagent F.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes²:

	<u>Test</u>	<u>Blank</u>
Reagent A (React Cocktail)	2.80	2.80
Reagent E (PK/LDH)	0.05	0.05
Reagent C (ATP)	0.10	0.10
Reagent B (PEP)	0.10	0.10
Reagent G (CK)	0.10	-----
Reagent F (Enz Dil)	-----	0.10

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

Reagent D (CC)

0.10

0.10

**Enzymatic Assay of CHOLINE KINASE
(EC 2.7.1.32)**

PROCEDURE: (continued)

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:

$$\text{Units/ml enzyme} = \frac{(r A_{340\text{nm}}/\text{min Test} - r A_{340\text{nm}}/\text{min Blank})(3.25)(\text{df})}{(6.22)(0.1)}$$

3.25 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will catalyze the phosphorylation of 1.0 μ mole of choline to choline phosphate by ATP per minute at pH 8.5 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.25 ml reaction mix, the final concentrations are 86 mM glycylglycine, 52 mM potassium chloride, 9.1 mM magnesium chloride, 3 mM ethylenediaminetetraacetic acid, 0.2 mM β -nicotinamide adenine dinucleotide, reduced form, 1.5 mM phospho(enol)pyruvate, 0.9 mM adenosine 5'-triphosphate, 3.1 mM choline chloride, 35 units pyruvate kinase, 50 units lactic dehydrogenase, 0.3 mM Tris acetate, 0.4 mM 2-mercaptoethanol, and 0.025 - 0.05 unit choline kinase.

REFERENCE:

Wittenberg, J. and Kornberg, A. (1953) *Journal of Biological Chemistry* **202**, 431-444

**Enzymatic Assay of CHOLINE KINASE
(EC 2.7.1.32)**

NOTES:

1. Contains not less than 700 Pyruvate Kinase units and 1000 Lactic Dehydrogenase units per ml.
2. These reagents must be pipetted fresh for each set of samples, since the phospho(enol)pyruvate may break down in the reaction mixture and give a secondary reaction.
3. Pyruvate Kinase Unit Definition: One unit will convert 1.0 μ mole of phospho(enol)pyruvate to pyruvate per minute at 7.6 at 37°C.
4. L-Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 μ mole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.
5. This assay is based on the cited reference.
6. 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.