Enzymatic Assay of CHOLINE KINASE
(EC 2.7.1.32)

PRINCIPLE:

Choline + ATP $\xrightarrow{CK}$ o-Phosphocholine + ADP
ADP + PEP $\xrightarrow{PK}$ ATP + Pyruvate
Pyruvate + $\beta$-NADH $\xrightarrow{LDH}$ Lactate + $\beta$-NAD

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
CK = Choline Kinase
ADP = Adenosine 5'-Diphosphate
PEP = Phospho(enol)pyruvate
PK = Pyruvate Kinase
$\beta$-NADH = $\beta$-Nicotinamide Adenine Dinucleotide, Reduced Form
LDH = Lactic Dehydrogenase
$\beta$-NAD = $\beta$-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 $\beta$-Nicotinamide Adenine Dinucleotide, Reduced Form, pH 8.5 at 25°C (React Cocktail)

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.)
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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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (React Cocktail)</td>
<td>2.80</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent E (PK/LDH)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent C (ATP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent B (PEP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent G (CK)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Reagent F (Enz Dil)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the  
A₃₄₀nm until constant, using a suitably thermostatted  
spectrophotometer. Then add:
| Reagent D (CC) | 0.10 | 0.10 |
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PROCEDURE:  (continued)

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

CALCULATIONS:

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

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 µmole 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
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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 µmole 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 µmole 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.