Enzymatic Assay of KALLIKREIN
(EC 3.4.21.34)
from Human Plasma

PRINCIPLE:

BAEE $\xrightarrow{\text{Kallikrein}}$ Na-Benzoyl-L-arginine + Ethanol

Ethanol + $\beta$-NAD $\xrightarrow{\text{ADH}}$ Acetaldehyde + $\beta$-NADH

Abbreviations used:
BAEE = Na-Benzoyl-L-Arginine Ethyl Ester
$\beta$-NAD = $\beta$-Nicotinamide Adenine Dinucleotide, Oxidized Form
ADH = Alcohol Dehydrogenase
$\beta$-NADH = $\beta$-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS:  T = 25°C, pH = 8.7, $A_{340nm}$, Light path = 1 cm

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:
A. 335 mM Pyrophosphate, 197 mM Semicarbazide and 53 mM Glycine Buffer, pH 8.7 at 25°C

B. 30 mM $\beta$-Nicotinamide Adenine Dinucleotide, Oxidized Form, Solution ($\beta$-NAD)
(Dissolve the contents of one 50 mg vial of $\beta$-Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-150, in the appropriate volume of deionized water. PREPARE FRESH.)

C. 6 mM Na-Benzoyl-L-Arginine Ethyl Ester Solution (BAEE)
(Prepare 10 ml in deionized water using Na-Benzoyl-L-Arginine Ethyl Ester, Hydrochloride, Sigma Prod. No. B-4500.)

D. 2.4 mM Ammonium Sulfate Solution $(\text{NH}_4)_2\text{SO}_4$
(Prepare 25 ml in deionized water using Ammonium Sulfate, Sigma Prod. No. A-5132.)
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REAGENTS: (continued)

E. Alcohol Dehydrogenase Enzyme Solution (ADH)  
(Immediately before use, prepare a solution containing  
10,000 units/ml of Alcohol Dehydrogenase, Sigma  
Prod. No. A-7011 in cold Reagent D.)

F. Kallikrein Solution  
(Immediately before use, prepare a solution containing  
0.15 - 0.30 unit/ml of Kallikrein in cold deionized  
water.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.30</td>
</tr>
<tr>
<td>Reagent B (β-NAD)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (BAEE)</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent E (ADH)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (Kallikrein)</td>
<td>0.10</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>------</td>
</tr>
</tbody>
</table>

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

CALCULATIONS:

$\text{Units/ml enzyme} = \frac{(ΔA_{340nm}/\text{min Test} - ΔA_{340nm}/\text{min Blank})(3.02)(df)}{(6.22)(0.1)}$

3.02 = 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

$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$
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CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of BAEE to Na-benzoyl-L-arginine and ethanol per minute at pH 8.7 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.02 ml reaction mix, the final concentrations are 255 mM pyrophosphate, 150 mM semicarbazide, 40 mM glycine, 1 mM Na-benzoyl-L-arginine ethyl ester, 1 mM β-nicotinamide adenine dinucleotide, 16 mM ammonium sulfate, 200 units alcohol dehydrogenase and 0.015 – 0.030 unit kallikrein.

REFERENCE:


NOTES:

1. Alcohol Dehydrogenase Unit Definition: One unit will convert 1.0 µmole of ethanol to acetaldehyde per minute at pH 8.8 at 25°C.

2. This assay is based on the cited reference.

3. 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.