

**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<sub>340nm</sub>, 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  
(Prepare 100 ml in deionized water using Pyrophosphate, Tetrasodium, Anhydrous, Sigma Prod. No. P-8010, Semicarbazide Hydrochloride, Sigma Prod. No. S-4125 and Glycine, Free Base, Sigma Prod, No. G-7126. Adjust to pH 8.7 at 25°C with 2 M NaOH.)
- 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 (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
(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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.30	2.30
Reagent B (β-NAD)	0.10	0.10
Reagent C (BAEE)	0.50	0.50
Reagent E (ADH)	0.02	0.02

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

Reagent F (Kallikrein)	0.10	-----
Deionized Water	-----	0.10

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

**CALCULATIONS:**

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

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  $\mu$ mole 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  $\beta$ -nicotinamide adenine dinucleotide, 16 mM ammonium sulfate, 200 units alcohol dehydrogenase and 0.015 - 0.030 unit kallikrein.

**REFERENCE:**

Trautschold, I., Werle, E. and Schweitzer, G. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Vol 2, 2nd ed., 1031 - 1034, Academic Press, Inc., New York, NY

**NOTES:**

1. Alcohol Dehydrogenase Unit Definition: One unit will convert 1.0  $\mu$ mole 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.**