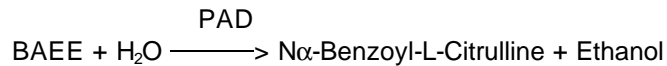


**Enzymatic Assay of PEPTIDYL ARGININE DEIMINASE
(EC 3.5.3.15)**

PRINCIPLE:



Abbreviation used:

PAD = Peptidyl Arginine Deiminase

CONDITIONS: T = 55°C, pH 7.2, $A_{490\text{nm}}$, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 350 mM Tris HCl Buffer, pH 7.2 at 55°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.2 at 55°C using 1 M HCl.)
- B. 70 mM Calcium Chloride Solution (CaCl_2)
(Prepare 10 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)
- C. 70 mM Benzoyl Arginine Ethyl Ester Solution (BAEE)
(Prepare 10 ml in deionized water using N- α -Benzoyl-L-Arginine Ethyl Ester, Hydrochloride, Sigma Prod. No. B-4500.)
- D. 70 mM DL-Dithiothreitol Solution (DTT)
(Prepare 10 ml in deionized water using DL-Dithiothreitol, Sigma Prod. No. D-0632. **Prepare Fresh.**)
- E. 60% (w/v) Perchloric Acid Solution (HClO_4)
(Prepare 100 ml in deionized water using Perchloric Acid, Sigma Stock No. 24425-2.)

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REAGENTS:

- F. Redox Reagent (Redox)
(Prepare by dissolving 11 g of Ferrous Ammonium Sulfate Hexahydrate, Sigma Prod. No. F-2262 and 9 g of Ammonium Iron (III) Sulfate Dodecahydrate, Aldrich Stock No. 22,126-0, in 100 ml of 1 N H₂SO₄. Heat gently with stirring for 30 minutes to get a clear solution. Keep in a plastic bottle covered with aluminum foil to protect from light.)
- G. Acid Mixture (Acid)
(Prepare 600 ml by adding 300 ml of Phosphoric Acid, Sigma Prod. No. P6560 to 200 ml of deionized water. Then add 100 ml of Sulfuric Acid, Sigma Prod. No. S-1526. Do not add water to acid!)
- H. 75 mM 2,3-Butanedione Monoxime Solution (BMO)
(Prepare 100 ml in deionized water using 2,3-Butanedione Monoxime, Sigma Prod. No. B-0753.)
- I. 1 mM Citrulline Standard Solution (Cit Std)
(Prepare 100 ml in deionized water using L-Citrulline. Sigma Prod. No. C-7629.)
- J. 0.1% (w/v) Bovine Serum Albumin Solution (Enz Dil)
(Prepare 10 ml in deionized water using Albumin, Bovine, Sigma Prod. No. A-4503.)
- K. Peptidyl Arginine Deiminase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 - 2.5 units per ml in cold Reagent J.)

PROCEDURE:

Pipette (in milliliters) the following reagents into an Eppendorf tube:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.20	0.20
Reagent B (CaCl ₂)	0.10	0.10
Reagent D (DTT)	0.10	0.10
Reagent K (Enz Sol)	0.20	-----
Reagent J (Enz Dil)	----- 0.20	

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PROCEDURE: (continued)

Preincubate for 2 minutes at 55°C. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent E (HClO ₄)	-----	0.10
Reagent C (BAEE)	0.10	0.10

Incubate for 30 minutes at 55°C. (Make sure the top of each tube is closed.) Then add:

Reagent E (HClO ₄)	0.10	-----
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Centrifuge to clarify.

Remove 0.4 ml of the supernatant from both the Test and Blank and place each into a glass tube.

Add 0.1 ml of Reagent F (REDOX) to each tube and mix by swirling. Boil for 10 minutes with the tubes covered with a glass onion. Remove and cool tubes.

Add 0.5 ml of Reagent G (Acid) and 0.2 ml of Reagent H (BMO) to both the Test and Blank. Mix on a vortexer. Boil for 20 minutes and then cool.

Transfer to suitable cuvettes and record the A_{490nm} for both the Test and Blank using a suitable spectrophotometer.

Standard Curve:

Prepare a standard curve by pipetting (in milliliters) the following reagents into suitable glass tubes.

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std Blank</u>
Reagent I (Cit Std)	0.050	0.100	0.200	-----
Deionized Water	0.35	0.30	0.200	0.40

Add 0.1 ml of Reagent F (REDOX) to each and mix on a vortexer. Boil for 10 minutes with the tubes covered with a glass marble. Remove and cool tubes.

Add 0.5 ml of Reagent G (Acid) and 0.2 ml of Reagent H (BHO) to the Standard and Standard Blank. Mix on a vortexer. Boil for 20 minutes and then cool.

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PROCEDURE: (continued)

Transfer to suitable cuvettes and record the $A_{490\text{nm}}$ for the Standards and Standard Blank, using a suitable spectrophotometer.

CALCULATIONS:

Calculate the absorbance of 1 μmole of citrulline, using the Standard curve.

$$\text{Units/ml} = \frac{(A_{490\text{nm}} \text{ Test} - A_{490\text{nm}} \text{ Blank})(2)(\text{df})}{(A_{490\text{nm}}/\mu\text{mole})(0.5)(0.1)}$$

2 = Conversion factor since only one-half of the reaction mixture is used in the colorimetric determination of citrulline

df = Dilution factor

$A_{490\text{nm}}/\mu\text{mole} = A_{490\text{nm}}/\mu\text{mole}$ of citrulline

0.5 = Conversion factor from 30 minutes to 1 hour

0.1 = Volume (in milliliters) 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 produce 1 μmole of N α -benzoylcitrulline ethyl ester from BAEE per hour at 55°C at pH 7.2.

FINAL ASSAY CONCENTRATION:

In a 0.7 ml reaction mix, the final concentrations are 100 mM Tris, 10 mM calcium chloride, 10 mM DL-dithiothreitol, and 0.1 - 0.5 unit peptidyl arginine deiminase.

REFERENCE:

Takahara, H., Okamoto, H., and Sugawara, K. (1986) *Journal of Biochemistry*, 99, 1417-1424

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NOTES:

1. This assay is based on the cited reference.
2. 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.