Enzymatic Assay of PEPTIDYL ARGinine DEIMINASE
(EC 3.5.3.15)

PRINCIPLE:

PAD

BAEE + H₂O → Nα-Benzoyl-L-Citrulline + Ethanol

Abbreviation used:
PAD = Peptidyl Arginine Deiminase

CONDITIONS: T = 55°C, pH 7.2, A₄₉₀nm 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, Adjust to pH 7.2 at 55°C using 1 M HCl.)

B. 70 mM Calcium Chloride Solution (CaCl₂)
(Prepare 10 ml in deionized water using Calcium Chloride, Dihydrate)

C. 70 mM Benzoyl Arginine Ethyl Ester Solution (BAEE)
(Prepare 10 ml in deionized water using N-α-Benzoyl-L-Arginine Ethyl Ester, Hydrochloride, Prepare Fresh.)

D. 70 mM DL-Dithiothreitol Solution (DTT)
(Prepare 10 ml in deionized water using DL-Dithiothreitol. Prepare Fresh.)

E. 60% (w/v) Perchloric Acid Solution (HClO₄)
(Prepare 100 ml in deionized water using Perchloric Acid)
Enzymatic Assay of PEPTIDYL ARGININE DEIMINASE
(EC 3.5.3.15)

REAGENTS:

F. Redox Reagent (Redox)
(Prepare by dissolving 11 g of Ferrous Ammonium Sulfate Hexahydrate, and 9 g of Ammonium Iron (III) Sulfate Dodecahydrate, in 100 ml of 1 N H$_2$SO$_4$. 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, to 200 ml of deionized water. Then add 100 ml of Sulfuric Acid. 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)

I. 1 mM Citrulline Standard Solution (Cit Std)
(Prepare 100 ml in deionized water using L-Citrulline.)

J. 0.1% (w/v) Bovine Serum Albumin Solution (Enz Dil)
(Prepare 10 ml in deionized water using Albumin, Bovine.)

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:

<table>
<thead>
<tr>
<th>Reagent A (Buffer)</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (CaCl$_2$)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent D (DTT)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent K (Enz Sol)</td>
<td>0.20</td>
<td>-----</td>
</tr>
<tr>
<td>Reagent J (Enz Dil)</td>
<td>-----</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Enzymatic Assay of PEPTIDYL ARGININE DEIMINASE (EC 3.5.3.15)

PROCEDURE: (continued)

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (HClO₄)</td>
<td>-----</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (BAEE)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (HClO₄)</td>
<td>0.10</td>
<td>-----</td>
</tr>
</tbody>
</table>

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 A₄₉₀nm 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.

<table>
<thead>
<tr>
<th></th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent I (Cit Std)</td>
<td>0.050</td>
<td>0.100</td>
<td>0.200</td>
<td>-----</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.35</td>
<td>0.30</td>
<td>0.200</td>
<td>0.40</td>
</tr>
</tbody>
</table>

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 (BMO) to the Standard and Standard Blank. Mix on a vortexer. Boil for 20 minutes and then cool.
Enzymatic Assay of PEPTIDYL ARGININE DEIMINASE (EC 3.5.3.15)

PROCEDURE: (continued)

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

CALCULATIONS:

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

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

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

df = Dilution factor

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

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 Nα-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:

Enzymatic Assay of PEPTIDYL ARGinine DEIMINASE
(EC 3.5.3.15)

NOTES:

1. This assay is based on the cited reference.

2. Where our Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of our quality control procedure contact our Technical Service Department.