Enzymatic Assay of TRANSGLUTAMINASE
(EC 2.3.2.13)

PRINCIPLE:

CBZ-Gln-Gly + Hydroxylamine $\xrightarrow{\text{Transglutaminase}}$ CBZ-Gln-Gly-Hydroxamate

Abbreviations used:
CBZ = N-Carbobenzoxy

CONDITIONS: $T = 37^\circ C$, pH = 6.0, $A_{525\text{nm}}$, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 1000 mM Tris Buffer, pH 6.0 at 37$^\circ$C
   (Prepare 50 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 6.0 at 37$^\circ$C with Glacial Acetic Acid.)

B. CBZ-Glutaminylglycine (CBZ-Gln-Gly)
   (Use Na-CBZ-Gln-Gly, Prod. No. C-6154.)

C. 200 mM Hydroxylamine with 20 mM Glutathione, Reduced Form Solution (HA/Glut)
   (Prepare 10 ml in deionized water using Hydroxylamine Hydrochloride, Prod. No. H-9876, and Glutathione, Reduced Form, Prod. No. G-4251. PREPARE FRESH.)

D. 1000 mM Calcium Chloride Solution (CaCl$_2$)
   (Prepare 1 ml in deionized water using Calcium Chloride Dihydrate, Prod. No. C-3881.)

E. 10 mM L-Glutamic Acid $\gamma$-Monohydroxamate Solution (Std)
   (Prepare 10 ml in deionized water using L-Glutamic Acid $\gamma$-Monohydroxamate, Prod. No. G-2253.)

F. 12% (v/v) Trichloroacetic Acid Solution (TCA)
   (Prepare 100 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, Stock No. 490-10)
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REAGENTS: (continued)

G. 5% (w/v) Ferric Chloride Solution (FeCl₃)
(Prepare 100 ml in Reagent H using Ferric Chloride, Hexahydrate, Prod. No. F-2877.)

H. 100 mM Hydrochloric Acid
(Prepare 100 ml in deionized water using Hydrochloric Acid, Prod. No. H-7020.)

I. Transglutaminase Enzyme Solution
(Immediately before use, prepare a solution containing 2 units/ml of Transglutaminase in cold deionized water.)

PROCEDURE:

Prepare a reaction cocktail by combining the following reagents into a suitable container:

Reagent B (CBZ-Gln-Gly) 120 mg

Then add (in milliliters):

Reagent A (Buffer) 2.00
Reagent C (HA/Glut) 5.00

Mix by inversion. Then add:

Reagent D (CaCl₂) 0.05

Mix by inversion. Adjust to pH 6.0 at 37°C with 100 mM NaOH. Then add enough deionized water to make a final volume of 10.0 ml.

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Test</th>
<th>Std.</th>
<th>Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>0.20</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Then add:
Reagent I (Enzyme Solution) 0.03 ------ ------ ------
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PROCEDURE: (continued)

Mix by inversion and incubate at 37°C for exactly 10 minutes. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Test Blank</th>
<th>Std.</th>
<th>Std Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>0.10</td>
</tr>
<tr>
<td>Reaction Cocktail</td>
<td>-----</td>
<td>0.20</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>Reagent E (Std)</td>
<td>-----</td>
<td>0.10</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Reagent F (TCA)</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent I (Enzyme Solution)</td>
<td>-----</td>
<td>0.03</td>
<td>-----</td>
<td></td>
</tr>
</tbody>
</table>

Mix by inversion. Then add:

Reagent G (FeCl₃) 0.50 0.50 0.50 0.50

Mix by inversion. Centrifuge for 5 minutes. Transfer the solutions to suitable cuvettes. Record the A₅₂₅nm for the Standard, Test and Blanks.

CALCULATIONS:

\[ E_{nm}^1 = \frac{(A_{525nm} Std. - A_{525nm} Std. Blank)}{(A_{525nm} Test - A_{525nm} Test Blank)} \times \frac{1}{(1.23)} \]

Units/mg enzyme = \( \frac{E_{nm}}{(mg \text{ enzyme/RM})} \times \frac{1}{(10)} \)

1.1 = Volume of Standard (in milliliters)
1.23 = Volume of Color Mix
RM = Reaction Mix (volume = 0.23 ml)
10 = Time of reaction in minutes

UNIT DEFINITION:

One unit of enzyme will catalyze the formation of 1.0 µmole of hydroxamate per minute from Na-CBZ-
Glutaminylglycine and hydroxylamine at pH 6.0 at 37°C. (L-Glutamic acid γ-monohydroxamate is the standard.)
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FINAL ASSAY CONCENTRATIONS:

In a 0.23 ml reaction mix, the final concentrations are 174 mM Tris, 31 mM CBZ-glutaminyglycine, 87 mM hydroxylamine, 8.7 mM glutathione, reduced form, 4 mM calcium chloride and 0.06 unit transglutaminase.

REFERENCES:


NOTES:

1. There may be lot to lot variation in the extinction coefficient of L-glutamic acid β-monohydroxamate; therefore, an extinction coefficient must be calculated for each lot. This calculation is based on reading the absorbance of a 1.1 ml standard solution which contains 0.1 ml of Reagent E (Std).

2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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