SIGMA QUALITY CONTROL TEST PROCEDURE

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

METHOD: Colorimetric

REAGENTS:

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

B. CBZ-Glutaminylglycine (CBZ-Gln-Gly)
(Use Nα-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 γ-Monohydroxamate Solution (Std)
(Prepare 10 ml in deionized water using L-Glutamic Acid γ-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)
Enzymatic Assay of TRANSGLUTAMINASE  
(EC 2.3.2.13)

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>Test</th>
<th>Blank</th>
<th>Std.</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>0.20</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Equilibrate to 37°C. Then add:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent I (Enzyme Solution)</td>
<td>0.03</td>
<td>------</td>
<td>------</td>
</tr>
</tbody>
</table>
Enzymatic Assay of TRANSGLUTAMINASE  
(EC 2.3.2.13)

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>Blank</th>
<th>Std.</th>
<th>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>-----</td>
<td>0.10</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 $A_{525\text{nm}}$ for the Standard, Test and Blanks.

CALCULATIONS:

$$E_{\text{nm}} = (A_{525\text{nm}} \text{ Std.} - A_{525\text{nm}} \text{ Std. Blank}) (1.1)$$

$$\text{Units/mg enzyme} = \frac{(A_{525\text{nm}} \text{ Test} - A_{525\text{nm}} \text{ Test Blank}) (1.23)}{(E_{\text{nm}}) (\text{mg enzyme/RM}) (10)}$$

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 Nα-CBZ-Glutaminylglycine and hydroxylamine at pH 6.0 at 37°C. (L-Glutamic acid γ-monohydroxamate is the standard.)
Enzymatic Assay of TRANSGLUTAMINASE  
(EC 2.3.2.13)

FINAL ASSAY CONCENTRATIONS:

In a 0.23 ml reaction mix, the final concentrations are 174 mM Tris, 31 mM CBZ-glutaminylglycine, 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 γ-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.

Sigma warrants that the above procedure information is currently utilized at Sigma and that Sigma products conform to the information in Sigma publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of Sigma products, see reverse side of invoice or packing slip for additional terms and conditions of sale.