3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-521-8956 • (314) 771-5765
Fax 800-325-5052 • (314) 771-5757
sigma-aldrich.com

ProductInformation

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°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)

REAGENTS: (continued)

G. 5% (w/v) Ferric Chloride Solution ($FeCl_3$)
(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 ($CaCl_2$)	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:

	Test	Test Blank	Std.	Std. Blank
Reaction Cocktail	0.20	------	------	------
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:

	Test	Test Blank	Std.	Std. Blank
Deionized Water	------	------	------	0.10
Reaction Cocktail	------	0.20	------	------
Reagent E (Std)	------	------	0.10	------
Reagent F (TCA)	0.50	0.50	0.50	0.50
Reagent I (Enzyme Solution)	------	0.03	------	------

Mix by inversion. Then add:

Reagent G ($FeCl_3$)	0.50	0.50	0.50	0.50

Mix by inversion. Centrifuge for 5 minutes. Transfer the solutions to suitable cuvettes. Record the A_{525nm} for the Standard, Test and Blanks.

CALCULATIONS:

$$E_{mM}^{1} = (A_{525nm} \text{ Std.} - A_{525nm} \text{ Std. Blank}) (1.1)$$

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

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 N_α-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-glutaminylglycine, 87 mM hydroxylamine, 8.7 mM glutathione, reduced form, 4 mM calcium chloride and 0.06 unit transglutaminase.

REFERENCES:

Folk, J. E. and Cole, P. W. (1966) *Biochim. Biophys. Acta* **122**, 244.

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.