

**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 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 (CaCl₂)
(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)

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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
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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:

	<u>Test</u>	<u>Test Blank</u>	<u>Std.</u>	<u>Std. Blank</u>
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
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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 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:

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.

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