Transglutaminase Activity Assay Kit (Colorimetric) (BN00800)

(Catalog # BN00800; 100 assays; Store at -20°C)

I. Introduction:

Transglutaminases (EC 2.3.2.13) are calcium dependent enzymes that catalyze the post-translational modification of proteins by formation of isopeptide bonds. This occurs either through protein cross-linking via formation of γ-glutamyl-ε-lysine bonds or through incorporation of primary amines at selected peptide-bound glutamine residues. The transglutaminase enzyme family comprises the intracellular forms (TG1, TG3 and TG5) expressed mostly in the epithelial tissue; TG2 which is both intracellular and extracellular and expressed in various tissue types; TG4 which is expressed in the prostate gland; factor XIII which is expressed in blood; TG6 and TG7, whose tissue distribution is unknown and band 4.2 (lacking enzymatic activity) which is present on erythrocyte membranes. Transglutaminases also exhibit GTPase, phosphodiesterase and protein kinase activity. Transglutaminases are associated with certain neurological and autoimmune disorders and also cancer. Assay Genie's Transglutaminase Activity Assay kit utilizes the deamidation reaction of the transglutaminase enzyme with a donor and acceptor substrate resulting in the formation of a hydroxamate product. The hydroxamate product reacts with the Stop Solution forming a purple complex that can be measured colorimetrically at 525 nm. The limit of quantification of this assay is ~10 µU or 80 ng of recombinant hTG2 enzyme.

Donor Substrate + Acceptor Substrate —(Transglutaminase)→ Hydroxamate product + Ammonia

Hydroxamate product + Stop Solution → Purple Complex (OD 525 nm)

II. Applications:

Quantification of Transglutaminase enzyme activity

III. Sample Type

- Cell lysate
- Tissue lysate
- Recombinant Transglutaminase

IV. Kit Contents:

Components	BN00800	Cap Code	Part Number
TG Assay Buffer	12 ml	WM	BN00800-1
Homogenization Buffer (10x)	10 ml	NM Clear	BN00800-2
1M DTT	125 µl	Blue	BN00800-3
Donor Substrate	1 Vial	NM Brown	BN00800-4
Acceptor Substrate	2 Vials	Red	BN00800-5
Hydroxamate Standard	1 Vial	Yellow	BN00800-6
Stop Solution	5 ml	NM Red	BN00800-7
Positive Control	1 Vial	Brown	BN00800-8
Plate Sealer	1	-	BN00800-9

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom.
- Multi-well spectrophotometer capable of absorbance detection
- Protease Inhibitor Cocktail
- Homogenizer
- 20% glycerol in water.
- Phosphate Buffered Saline (PBS)
- Deionized water

VI. Reagent Preparation and Storage Conditions:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **TG Assay Buffer:** Warm to 37°C before use.
- **Homogenization Buffer:** Thaw and make 1x buffer by diluting 1 volume 10x Homogenization Buffer with 9 volumes deionized water. Keep on ice while in use.
- **1 M DTT:** Store at -20°C. Thaw and keep on ice while in use. Use within two months.
- **Donor Substrate:** Reconstitute with 1.1 ml deionized water**.** Aliquot and store at -80°C. Avoid repeated freeze/thaw. Use within two months.
- **Acceptor Substrate:** Reconstitute each vial with 550 µl deionized water as needed. Aliquot and store at -80°C. Use within two months.
- **Hydroxamate Standard:** Reconstitute with 330 µl deionized water to make 10 mM stock Standard solution**.**
- **Stop Solution:** Store at 4°C or -20°C. Thaw before use. Keep on ice while in use.
- **Positive Control:** Reconstitute with 30 µl 20% glycerol in water (not provided). Mix well, aliquot and store at - 80°C. Avoid repeated freeze/thaw. Thaw and mix gently before use.

VII. Transglutaminase Activity Assay Protocol:

1. **Sample Preparation**: Add DTT to 1x Homogenization Buffer at a final concentration of 0.2 mM. Make fresh as needed. Rinse tissue with PBS and transfer ~100 mg of fresh or frozen tissue (stored at -80°C) to a prechilled homogenizer. Add 500 µl cold Homogenization Buffer (with DTT) containing protease inhibitor cocktail (not provided) and thoroughly homogenize tissue on ice using a dounce or electrical homogenizer. To prepare cell extract, add 150-300 µl cold Homogenization Buffer (with DTT) containing protease inhibitor cocktail (not

provided) to 2-5 x 10^6 cells and disrupt cells by five cycles for freezing and thawing. Transfer the tissue or cell homogenate to a cold microfuge tube. Centrifuge the tissue or cell homogenate at 16,000 X g for 20 min. at 4°C. Transfer the clarified supernatant to a fresh pre-chilled tube and keep on ice. Prepare reaction in a 96-well clear plate as follows.

	Sample	Positive Control	Reagent Background Control
Lysate	25-50 µl	—	—
Positive Control	—	2 µl*	—
Deionized Water	Make up to 50 µl	48 µl	50 µl

Notes:

a) * Add Positive Control just before adding the Transglutaminase Reaction Mix (step 2).

b) To check the Transglutaminase activity, protein concentration of lysates should be at least 5 mg/ml.

c) We recommend using the lysates immediately to assay the Transglutaminase Activity.

d) Wash adherent cells with PBS. Add Homogenization Buffer (with DTT) containing protease inhibitor cocktail (not provided) to washed cells and collect cells by scraping into the buffer. Transfer cells in Homogenization Buffer to a prechilled tube and disrupt cells as described above. For suspension cells, directly add Homogenization Buffer (with DTT) containing protease inhibitor cocktail to the PBS washed cell pellet and resuspend. Disrupt cells as described above.

2. **Transglutaminase Reaction Mix:** Prepare 50 µl Reaction Mix for each Sample and Background Control as follows.

TG Assay Buffer	25 µl
Donor Substrate	10 µl
Acceptor Substrate	10 µl
1M DTT	1 µl
Deionized water	4 µl

Mix and add 50 µl to each well. Mix well. Incubate the reaction at 37°C for two hrs.

3. **Hydroxamate Standard:** To prepare Hydroxamate Standard, dilute TG Assay Buffer 1:1 with Deionized water. Add 0, 2, 4, 6, 8, and 10 µl of 10 mM Hydroxamate Standard into a series of wells in a **new** 96-well clear plate and make up the volume to 50 µl/well using the diluted TG Assay Buffer.

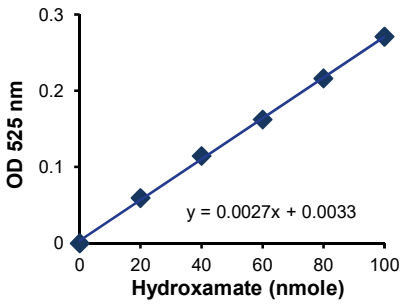
4. **Measurement:** Add 50 µl/well of the Stop Solution to all Standards and sample wells. Mix well by pipetting. Seal the sample plate, and centrifuge at ~1800x g for 15 min. to pellet the precipitate formed. Carefully transfer 100 µl of supernatant from all Sample and Reagent background Control wells into desired wells in the 96-well clear plate containing the Standards. Measure absorbance (OD 525 nm). Note: Standard will not precipitate after adding stop solution.

5. **Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the Standard Curve. Subtract Reagent Background Control reading from all sample readings. Apply sample's ΔRFU to the Standard Curve to get B nmoles of hydroxamate generated by sample in two hrs. in 100 µl reaction volume. Transglutaminase Activity is linear for the first 20 min.

Sample's Transglutaminase Activity = Bx1.5/T/mg = nmol/T/mg

Where: **B**: hydroxamate amount from Standard Curve (nmol).
1.5: nmoles of hydroxamate product generated in 150 µl reaction volume.
T: incubation time in minutes.
Mg: amount of protein/reaction in mg

Unit Definition: one unit of Transglutaminase activity is defined as the amount of enzyme that generates 1 µmole of the hydroxamate product per min. under the kit assay conditions.



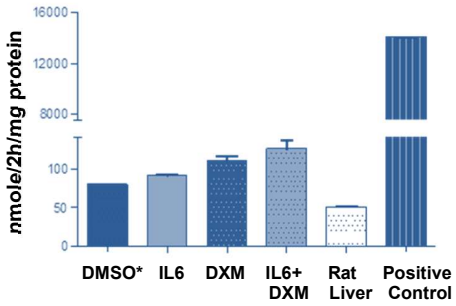


Figure (a) Hydroxamate Standard Curve. (b) Transglutaminase activity in HepG2 cells (human hepatoblastoma cell line) and rat liver lysate: HepG2 cells were stimulated with vehicle (DMSO), IL6 (1 µM), Dexamethasone (DXM -1 µM), or with IL6 (1µM) and DXM (1 µM). Approximately 250 µg protein was used for determining transglutaminase activity in cells and tissue lysate. Activity is expressed as nmoles of product formed in 2h and is normalized to the protein amount. Assays were performed following the kit protocol. *HepG2 cells have similar intrinsic Transglutaminase activity in the presence or absence of vehicle control (DMSO).

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