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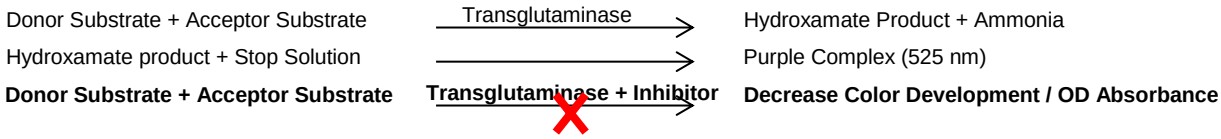
Transglutaminase Inhibitor Screening Assay Kit

6/16

(Catalog # K508-100; 100 assays; Store kit at -20°C)

I. Introduction:

Transglutaminases (TGs) are enzymes that catalyze post-translational modifications of glutamine residues in proteins, via the formation of isopeptide bonds, esterification and deamination. The reaction produces insoluble macromolecular complexes and protein aggregates. Within the TG family of enzyme, tissue-specific Transglutaminase-2 (TG2) expression is proven to be a clinical target for multiple types of cancer and neurodegenerative diseases, including Alzheimer's and Huntington's diseases. Inhibition of TG2, with a Cystamine-derived drug candidate (currently in phase-II clinical trial for Huntington's disease), has shown to have neuroprotective effects in models of neurodegeneration. Further, TG2 interacts with retinoblastoma protein (Rb), GTPase, and protein kinases through serine/threonine phosphorylation in cancer. Therefore, TG2 is a clinical validated candidate in these pathological diseases, and is a highly relevant therapeutic biomarker. BioVision's Transglutaminase Inhibitor Screening Assay Kit utilizes the catalytic activity of TG2, in a deamidation reaction between donor and acceptor substrates, to generate a hydroxamate product. The stop solution reacts with the hydroxamate product, forming a purple complex that can be measured at 525 nm. In the presence of TG2 inhibitor, the enzyme loses its ability to catalyze the deamidation reaction, leading to a decrease in color development and a lower absorbance OD reading. This assay kit is simple and can be used to identify and characterize TG2 inhibitors in a high-throughput format.



II. Application:

- Screening/ characterizing/ studying TG2 inhibitors

III. Kit Contents:

Components	K508-100	Cap Code	Part Number
TG Assay Buffer	12 ml	WM	K508-100-1
1 M DTT	110 µl	Blue	K508-100-2
Donor Substrate (lyophilized)	1 bottle	NM brown	K508-100-3
Acceptor Substrate (lyophilized)	1 bottle	NM Red	K508-100-4
Stop Solution	8 ml	NM Clear	K508-100-5
Transglutaminase-2 Enzyme (lyophilized)	1 vial	Violet	K508-100-6
Inhibitor Cystamine (lyophilized)	1 vial	Green	K508-100-7

IV. User Supplied Reagents and Equipment:

- 96-clear well plate with flat bottom
- Microplate reader
- 20 % glycerol in water
- Deionized water

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Avoid repeated freeze/thaw for all non-buffer components. Briefly centrifuge small vials before opening. Read entire protocol before performing the assay.

- **TG Assay Buffer:** Warm to 37°C before use.
- **1 M DTT:** Store at -20°C. Thaw and keep on ice while in use. Use within 6 months.
- **Donor Substrate (lyophilized):** Reconstitute with 1.65 ml deionized water. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Use within 3 months.
- **Acceptor Substrate (lyophilized):** Reconstitute with 1.65 ml deionized water. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Use within 3 months.
- **Stop Solution:** Store at 4°C or -20°C. Thaw and keep on ice while in use.
- **Transglutaminase-2 Enzyme (lyophilized):** Reconstitute enzyme with 1 ml of 20 % glycerol in water (not provided). Aliquot and store at -80°C. The enzyme stock solution is stable for up to 3 months in storage condition. Avoid repeated freeze/thaw. Enzyme stock is ready to use for procedure section VI, step 2.
- **Inhibitor Cystamine (lyophilized):** Prior to opening the vial, allow it to equilibrate to room temperature. Reconstitute the compound with 220 µl deionized water to make 1 M stock solution. Avoid repeated freeze/thaw. Store at -20 °C as aliquots. Use within 3 months.

VI. Transglutaminase Inhibitor Screening Assay Protocol:

1. **Inhibitor preparation:** Dissolve test compounds in appropriate solvent to produce stock solutions. For each test compound, prepare 10X solution of each desired test concentration.

   Note: High solvent concentration might affect enzyme activity. Keep the solvent final concentration less than 10% of 150 µl total reaction mix. DMSO at the concentration of 10% or less does not affect enzymatic activity. Prepare solvent control as needed.

To obtain 300 mM working solution of positive inhibitor control Cystamine (10X final concentration), add 30 µl of the 1 M stock solution to 70 µl of deionized water. Working solution should be prepared and used on the same day.

2. **Enzyme and Inhibitor Reaction Preparation:**

Prepare the TG2 enzyme, inhibitors and controls, as explained in the table below, for the following reaction conditions: Background Control [**BC**], Enzyme only Control [**EC**], Solvent Control [**SC**], Positive inhibitor Control [**PC**], Test Compound [**TC**].

Make 75 µl for each reaction and pre-incubate the TG2 enzyme and inhibitors or controls for 15 minutes at 37°C.

	BC	EC	SC	PC	TC
TG2 Enzyme	–	10 µl	10 µl	10 µl	10 µl
Solvent solution only	15 µl	–	15 µl	–	–
Test compound solution (10x)	–	–	–	–	15 µl
Inhibitor Cystamine (10x)	–	–	–	15 µl	–
Deionized Water	60 µl	65 µl	50 µl	50 µl	50 µl

3. **Substrate Preparation:** Make 75 µl substrate master mix for each well as below:

TG Buffer	38 µl
Donor Substrate	15 µl
Acceptor Substrate	15 µl
1 M DTT	1 µl
Deionized water	6 µl

Mix and add 75 µl of substrate master mix to all wells (**BC**, **EC**, **SC**, **PC**, **TC**), yielding a final reaction volume of 150 µl / well.

Incubate the reaction at 37°C for at least 1 hour (we recommend two-hour incubation for higher production of Hydroxamate product and darker color development).

4. **Stop Solution:** Add 75 µl of the Stop Solution to each well to stop the reaction and develop color. Mix thoroughly by pipetting, but avoid forming bubbles during mixing.
   Note: Reactions may appear cloudy at first, but should clear up within a few minutes.
5. **Measurement:** Read the absorbance OD at 525 nm in microplate reader as end-point mode.
6. **Calculation:** Subtract the Background Control reading from all readings to obtain ΔOD 525 for each reading. Set the ΔOD of Enzyme Control [EC] as 100%, and calculate % Inhibition or % Relative Activity of the test compound [TC] as follows:

$$\% \text{ Inhibition} = \frac{\Delta OD \text{ of EC} - \Delta OD \text{ of TC}}{\Delta OD \text{ of EC}} \times 100$$

$$\% \text{ Relative Activity} = \frac{\Delta OD \text{ of TC}}{\Delta OD \text{ of EC}} \times 100$$

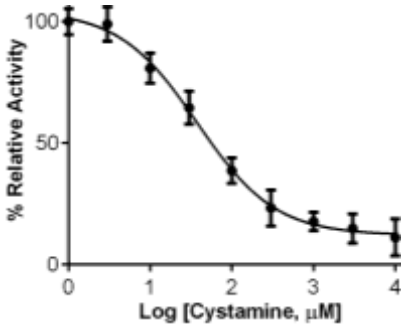


Figure: Inhibition of TG2 Enzyme activity by Cystamine. Cystamine was pre-incubated with TG2 enzyme for 15 minutes at 37°C. Substrate was added and incubated for 2 hours at 37°C. Assays are performed following the kit protocol. IC_{50} of Cystamine was determined to be 37.5 ± 2.6 µM. Assays are performed following the kit protocol.

VII. Related Products:

Transglutaminase Activity Assay Kit (K571-100)
AHCY Activity Fluorometric Assay Kit (K807)
NNMT Inhibitor Screening Kit (K822)
Transglutaminase Human Recombinant Enzyme (7700-100)
Active AHCY, human recombinant (7527)
Nicotinamide N-Methyltransferase (7261)

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