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Product Information

Transglutaminase Assay Kit

Catalog Number **CS1070**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Transglutaminases (TGases) are a widely distributed and unique group of calcium dependent enzymes that catalyze the post-translational modification of proteins by the formation of isopeptide bonds. This occurs either through protein crosslinking via formation of γ-glutamyl-ε-lysine bonds or through incorporation of primary amines at selected peptide-bound glutamine residues. The crosslinked products, often of high molecular mass, are highly resistant to mechanical challenge and proteolytic degradation, and their accumulation is found in a number of tissues and processes, where such properties are important including skin, hair, blood clotting, and wound healing.[1,2] Deregulation of the enzyme activity contributes to a number of human diseases such as inflammatory diseases (wound healing, tissue repair, and fibrosis), autoimmune diseases (including celiac disease, Type I diabetes, etc.), chronic degenerative diseases (rheumatoid arthritis and osteoarthritis), neurodegenerative diseases (senile dementia of the Alzheimer type, Huntington`s disease, rubropallidal atrophy, and spinocerebellar palsy), infectious diseases (Hepatitis C and AIDS), and cancer.

The kit is designed for detection of transglutaminase activity in biological samples. The kit can also be used for screening of transglutaminase inhibitors/activators.

The assay is based on transglutaminase catalysis of a covalent bond formation between a free amine group of poly-L-lysine, which is covalently attached to the plate surface, and the γ-carboxamide group of biotin-TVQQEL-OH substrate present in the Assay Buffer. This reaction immobilizes biotin to the plate surface. The amount of immobilized biotin is proportional to the amount of active transglutaminase in the sample. The amount of immobilized biotin is determined using Streptavidin−Peroxidase and TMB substrate.[3,4]

Components

The kit is sufficient for assays in two 96 well plates. Stripwell plates are supplied for the customer convenience.

Assay Buffer Catalog Number A5731	2.5 ml
Substrate Coated 96-well Plate Catalog Number S3076	2 each
DTT, 1 M Catalog Number D7059	400 µl
Transglutaminase from guinea pig liver (1 unit/vial) EC 2.3.2.13 Catalog Number T5398	2 each
3,3′,5,5′-Tetramethylbenzidine (TMB) Liquid Substrate System Catalog Number T0440	50 ml
Stop Solution Catalog Number S6697	25 ml
Streptavidin−Peroxidase from *Streptomyces avidinii* Catalog Number S5512	100 µg

Reagents and Equipment Required but Not Provided

- Ultrapure water
- Plate reader
- Phosphate buffered saline, with 0.05% TWEEN® 20 (PBS-T), Catalog Number P3563
- EDTA, Catalog Number E7889

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Use ultrapure water (17 MΩ·cm or equivalent) for the preparation of reagents and throughout the procedure.

Transglutaminase Stock Solution – Prepare a solution of 10 mM DTT and 1 mM EDTA. Dissolve the contents of one vial in 0.5 ml of this solution. The enzyme is stable for 2 weeks when stored in solution at 2–8 °C. **Do not aliquot and freeze the Transglutaminase Stock Solution**.

Positive Control Solution – **Just prior to the assay**, dilute 1 µl of the Transglutaminase Stock Solution in 1 ml of ultrapure water (final concentration of the enzyme is 2 milliunits/ml).

Streptavidin-Peroxidase Solution – Dissolve the Streptavidin−Peroxidase (Catalog Number S5512) vial contents in 100 µl of ultrapure water. Aliquot and store at –20 °C for up to 1 year. Avoid multiple freeze-thaw cycles.

Assay Mixture - For a single reaction, add 10 µl of the Assay Buffer (Catalog Number A5731) and 1 µl of 1 M DTT (Catalog Number D7059) to 40 µl of ultrapure water. For multiple assays, calculate the volume of Assay Mixture required. Prepare immediately before use.

Storage/Stability
The kit is shipped on dry ice and storage at –20 °C is recommended. Upon arrival, the Stop Solution can be stored at room temperature and the 3,3′,5,5′-Tetra-methylbenzidine (TMB) Liquid Substrate System (Catalog Number T0440) should be stored at 2–8 °C.

Procedure
These instructions are for a single reaction in a single well. For multiple reactions calculate accordingly.
Note: In order to compare relative activities between samples it is recommended to assay them on the **same** plate.

Table1.
Reaction scheme

Sample	Enzyme	Water	Assay Mixture
Blank	–	50 µl	50 µl
Test	x* µl	50–x* µl	50 µl
Positive Control	50 µl of Positive Control Solution	–	50 µl

x***** – volume of test sample added to the reaction.

1. Remove the substrate coated plate from the bag and allow it to equilibrate to room temperature. Unused strips should be stored with the desiccant pack in the tightly closed bag at –20 °C.
2. Add 50 µl of the Positive Control Solution to the Positive Control well.
3. Add 1–50 µl of sample to the Test well. Use a sample containing 1–10 µg of total protein (for cell or tissue lysates). Adjust the sample volume to 50 µl with ultrapure water.
4. Add 50 µl of Assay Mixture to each well. Mix by gentle finger tapping on the plate. Incubate on ice for 1–2 hours or at room temperature for 15–30 minutes.
5. While incubating, dilute 0.1 µl of the Streptavidin-Peroxidase Solution in 100 µl of PBS-T, for each well. Calculate the volume required for the total number of assays.
6. Wash the wells 3 times with ultrapure water.
7. Add 100 µl/well of freshly prepared Streptavidin-Peroxidase conjugate (step 5) and incubate for 20 minutes at room temperature.
8. Wash the wells no more than 3 times with 200 µl/well of PBS-T. Do not incubate with PBS-T, just wash.
9. Add 200 µl/well of 3,3′,5,5′-Tetramethylbenzidine (TMB) Liquid Substrate System, Catalog Number T0440. Stop the color development with 100 µl/well of Stop Solution (Catalog Number S6697) as soon as light blue color is observed. If light blue color is not observed, add Stop Solution after 1–3 minutes incubation at room temperature.
10. Read the absorption at 450 nm.

Inhibition reaction assay
The kit can be used to assay enzyme inhibitors. For performing an inhibition reaction, first optimize the concentration of the enzyme to be used. After step 1, add 1–25 µl of enzyme/sample and 1–25 µl inhibitor of choice to the appropriate wells. Adjust the volume to 50 µl with ultrapure water. Pre-incubate the enzyme with the inhibitor for 1–5 minutes. Continue from step 4 and on.

References

1. Chung, S.I. et al., Mechanism of action of guinea pig liver transglutaminase. VII. Chemical and stereochemical aspects of substrate binding and catalysis. J. Biol. Chem., **245**, 6424-6435 (1970).
2. Griffin, M. et al., Transglutaminases: Nature's biological glues. Biochem. J., **368**, 377-396 (2002).
3. Korner, G. et al., Bovine aortic endothelial cell transglutaminase. Enzyme characterization and regulation of activity. Biochem. J., **262**, 633-641 (1989).
4. Facchiano, F., and Facchiano, A., Trans-glutaminases and their substrates. Prog. Exp. Tumor Res., **38**, 37-57 (2005).

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