Transglutaminase Activity Assay Kit

Item No. 702250

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1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. After opening kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
400540	Transglutaminase Assay Substrate 1	2 vials	-20°C
400541	Transglutaminase Assay Substrate 2	1 vial/200 µl	-20°C
400542	Transglutaminase Assay Stop Solution	1 vial/10 ml	4°C
400543	Transglutaminase Assay Positive Control	1 vial/90 µl	-80°C
700020	Half Volume 96-Well Clear Plate	1 plate	RT
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 525 nm
2. An orbital microplate shaker
3. An incubator capable of heating a plate at 37°C
4. Adjustable pipettes; multichannel or repeating pipettor recommended
5. A source of pure water; glass-distilled water or pure water is acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*

INTRODUCTION

Background

Transglutaminases (TGs) are calcium-dependent enzymes involved in post-translational modifications of proteins or peptides and various cellular and biological processes such as blood clotting, apoptosis, cell differentiation, and extracellular matrix stabilization.[1-3] The human transglutaminase family is composed of eight catalytically active members, TG1-7 and Factor XIIIa, and one catalytically inactive member, erythrocyte-bound 4.2, which lacks an active site cysteine residue.[3] Like cysteine proteases, the TG catalytic mechanism involves a triad of Cys-His-Asp or Cys-His-Asn and forms an acyl-enzyme intermediate, which can form isopeptide bond cross-links between proteins or peptides, conjugate small molecules such as polyamines to proteins, or deamidate glutamine to form glutamate.[2,3] Some TGs possess additional catalytic activities, including GTPase and serine/threonine kinase activities.[1] The most well-studied isoform, TG2, is ubiquitously expressed and localizes to the intra- and extracellular spaces, whereas other isoforms are tissue-specific and localize to cellular and subcellular compartments.[4] TG2 acts on diverse protein substrates, including the cytoskeletal proteins tau and myosin, extracellular matrix-associated proteins collagen and fibronectin, and signaling proteins substance P and phospholipase A_2 (PLA_2), as well as exogenous proteins such as *C. albicans* surface proteins and HIV gp120.[3,4] Dysregulation of TG2 is associated with many neurodegenerative, autoimmune, dermatological, and endocrine diseases.[4]

About This Assay

Cayman's Transglutaminase Activity Assay Kit provides a convenient method of detecting transglutaminase activity in cell lysates and tissue homogenates. In this assay, transglutaminase transfers an amine group from hydroxylamine (amine donor, Transglutaminase Assay Substrate 2) to a glutamine residue of a peptide substrate (amine acceptor, Transglutaminase Assay Substrate 1). The resulting glutamyl-hydroxamate-containing product forms a chromogenic complex with ferric iron (Fe^{3+}), which is readily detectable at 525 nm (see Figure 1). With our Transglutaminase Assay Substrate 1, transglutaminases have a reaction rate that is 16-fold faster when compared to the traditional amine acceptor, Z-Gln-Gly, as the substrate. Thus, Cayman's Transglutaminase Activity Assay kit allows for achieving significantly higher signals in a shorter, 20-minute reaction time. Furthermore, the Transglutaminase Assay Stop Solution is specially formulated to prevent the aggregation of proteins in most samples, allowing users to avoid a centrifugation step that is required in many competitors' assay kits.

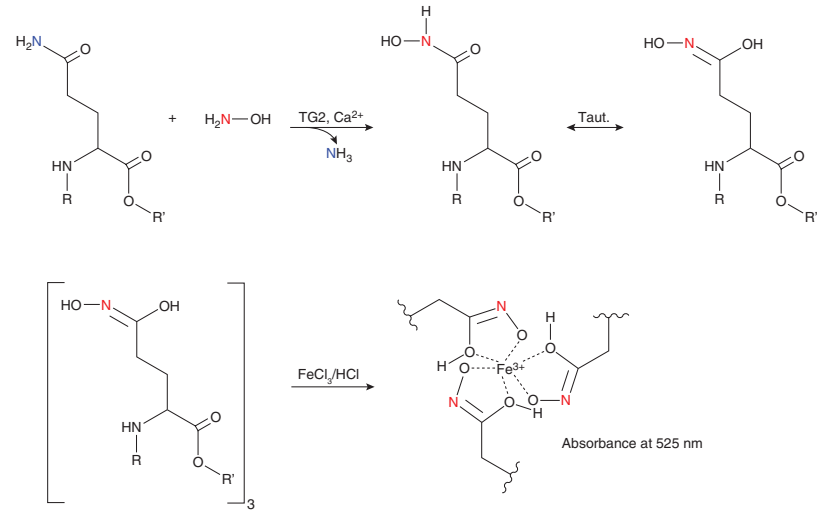


Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Transglutaminase Assay Substrate 1 – (Item No. 400540)**

   Each vial contains a lyophilized powder of Transglutaminase Assay Substrate 1. Immediately prior to assaying, reconstitute the entire contents of one vial with 2 ml of water. Mix thoroughly until completely dissolved. This will make a volume of Transglutaminase Assay Substrate 1 sufficient for 50 wells. Reconstitute two vials if running a full plate. The reconstituted Transglutaminase Assay Substrate 1 will be stable for 24 hours when stored at 4°C and for at least 6 months when stored at -20°C.

2. **Transglutaminase Assay Substrate 2 – (Item No. 400541)**

   This vial contains 200 µl of a concentrated Transglutaminase Assay Substrate 2 solution. Mix 150 µl of Transglutaminase Assay Substrate 2 with 1.35 ml of pure water. This is a volume of substrate sufficient for an entire 96-well plate. This solution will be stable for at least 6 months when stored at -20°C.

3. **Transglutaminase Assay Stop Solution – (Item No. 400542)**

   This vial contains 10 ml of Transglutaminase Assay Stop Solution. The reagent is ready to use as supplied. This reagent will be stable for at least 6 months when stored at 4°C.

4. **Transglutaminase Assay Positive Control – (Item No. 400543)**

   This vial contains 90 µl of recombinant human transglutaminase 2. It is ready to use as supplied. This solution will be stable for at least 6 months when stored at -80°C. However, it is advised to aliquot into smaller batches and avoid multiple freeze-thaws when not using all at once.

Sample Preparation

Tissue Homogenate

1. Prior to dissection, rinse the tissue with PBS, pH 7.4, to remove any red blood cells and clots. *NOTE: Hemolysis interferes with this assay and reduces the accuracy of the results, even when proper sample background wells are included. It is important to rinse the tissue with PBS well before dissection and homogenization.*
2. Homogenize the tissue in 5-10 ml of cold buffer per gram of tissue. It is recommended that buffers such as T-PER™ or 100 mM HEPES, pH 7.6, containing 40 mM $CaCl_2$ and 10 mM DTT be used to maintain transglutaminase in its active form.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant and store on ice. If not assaying on the same day, store the sample at -20°C.

Cell Lysate

1. Collect cells (~5 x 10^6) by centrifugation (*i.e.*, 500-1,000 x g for 10 minutes at 4°C). For adherent cells, use a cell scraper instead of proteolytic enzymes.
2. Homogenize the cell pellet in 0.5-1 ml cold buffer. It is recommended that buffers such as M-PER™ or 100 mM HEPES, pH 7.6, containing 40 mM $CaCl_2$ and 10 mM DTT be used to maintain transglutaminase in its active form.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant and store on ice. If not assaying on the same day, store the sample at -80°C.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. It is recommended that two wells be designated for the positive control, each sample be assayed at least in duplicate, and that the contents of each well are recorded on the template sheet provided on page 25. A typical layout of samples to be measured in duplicate is provided below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW	BW	7	7	15	15	23	23	31	31	39	39
B	PC	PC	8	8	16	16	24	24	32	32	40	40
C	1	1	9	9	17	17	25	25	33	33	41	41
D	2	2	10	10	18	18	26	26	34	34	42	42
E	3	3	11	11	19	19	27	27	35	35	43	43
F	4	4	12	12	20	20	28	28	36	36	44	44
G	5	5	13	13	21	21	29	29	37	37	45	45
H	6	6	14	14	22	22	30	30	38	38	46	46

BW = Blank Wells
PC = Positive Control Wells
1-46 = Sample Wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that an multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 120 µl in all the wells.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended to assay the samples at least in duplicate.
- Up to 30 samples can be assayed in triplicate or 46 in duplicate.
- The assay is performed at 37°C.
- Monitor the absorbance at 525 nm.

Performing the Assay

1. **Positive Control Wells**

   Add 40 µl of reconstituted Transglutaminase Assay Substrate 1 and 10 µl of reconstituted Transglutaminase Assay Positive Control to the designated wells on the plate (see **Sample plate format**, Figure 2, page 10).

2. **Sample Wells**

   Add 40 µl of reconstituted Transglutaminase Assay Substrate 1 and 10 µl of sample to the designated wells on the plate.

3. **Blank Wells**

   Add 40 µl of reconstituted Transglutaminase Assay Substrate 1 and 10 µl of homogenate/lysis buffer to the designated wells on the plate.

4. **Sample Background Wells**

   The background activity is typically insignificant in the evaluation of transglutaminase activity in samples such as purified proteins and cell lysates. *However, it is critical to include sample background wells when using tissue homogenates.* For each sample being assayed, add 40 µl of reconstituted Transglutaminase Assay Substrate 1, 10 µl of homogenate/lysis buffer, and 10 µl of sample to the designated wells on the plate.

5. Cover the plate and incubate at 37°C for 5 minutes.

6. Remove the plate cover and quickly initiate the reactions by adding 10 µl of diluted Transglutaminase Assay Substrate 2 to the positive control wells, sample wells, and blank wells. Do not add the Transglutaminase Assay Substrate 2 to the sample background wells.

7. Cover the plate and incubate at 37°C for 20 minutes.

Well	Substrate 1	Positive Control	Sample	Homogenate /Lysis Buffer	Substrate 2
Positive Control	40 µl	10 µl	--	--	10 µl
Sample	40 µl	--	10 µl	--	10 µl
Blank	40 µl	--	--	10 µl	10 µl
Sample Background	40 µl	--	10 µl	10 µl	--

Table 1. Pipetting summary

8. Remove the plate cover and quickly stop the reactions by adding 60 µl of Transglutaminase Assay Stop Solution to all of the wells being used.

9. Mix thoroughly by briefly shaking or gently tapping the plate. Read absorbance at 525 nm.

Calculations

1. Determine the average absorbance values of the blank, sample background, positive control wells, and each of the sample wells.
2. Subtract the average absorbance values of the blank wells from the average absorbance values of the positive control wells. Subtract the average absorbance values of the sample background or blank wells from the average absorbance values of the corresponding sample wells. This is the corrected signal (CS).
3. Use the following formula to calculate the transglutaminase activity.

$$\text{activity (U/ml)} = \left[\frac{\text{CS x 0.12 ml}}{\text{0.01 ml x } \varepsilon \text{ x d x t}}\right] = \text{CS x 0.88 (µmol/min/ml)}$$

where,
ξ is the extinction coefficient = 0.9705 $mM^{-1}cm^{-1}$
d is the path length = 0.702 cm
t is the reaction time = 20 minutes

One U/ml of transglutaminase activity is defined as the amount of enzyme per ml of sample that catalyzes the conversion of 1 µmol of substrate per minute under the conditions used in this assay. To convert to U/mg, use the following conversion factor:

$$\text{U/mg} = \left[\frac{\text{U/ml}}{\text{sample concentration (mg/ml)}}\right]$$

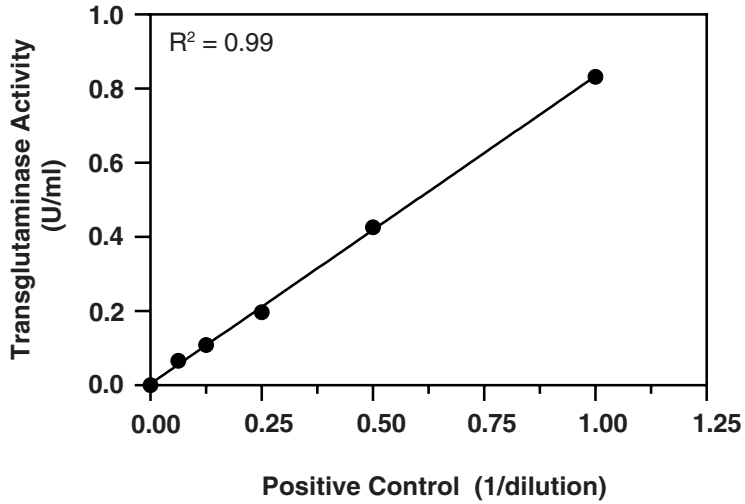


Figure 3. Activity of the Transglutaminase Assay Positive Control

Parallelism

Rat liver and brain tissues were homogenized as described in the Sample Preparation section, serially diluted with homogenate buffer, and evaluated using the Transglutaminase Activity Assay Kit. The results are shown below.

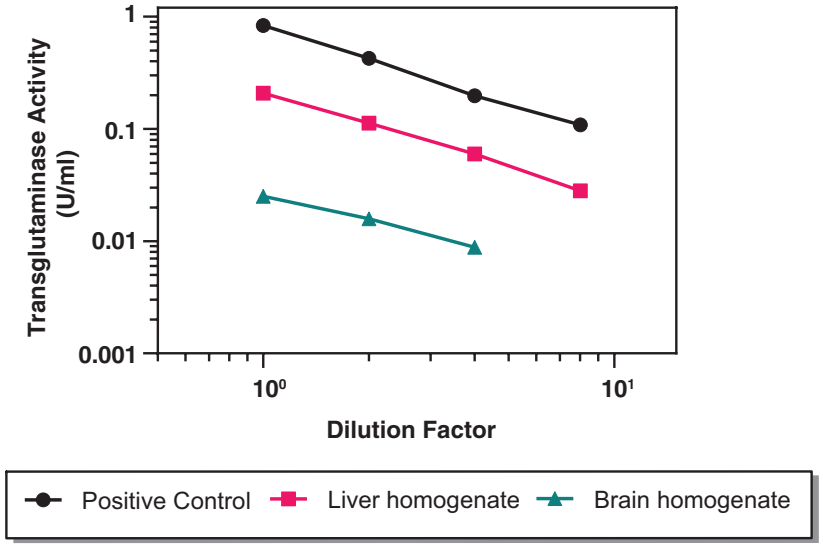


Figure 4. Parallelism of tissue samples in the Transglutaminase Activity Assay Kit

Spike and Recovery

Homogenates from rat liver and brain and HEK293 cell lysate were spiked with transglutaminase 2 (human, recombinant) and analyzed using the Transglutaminase Activity Assay Kit. The results are shown below.

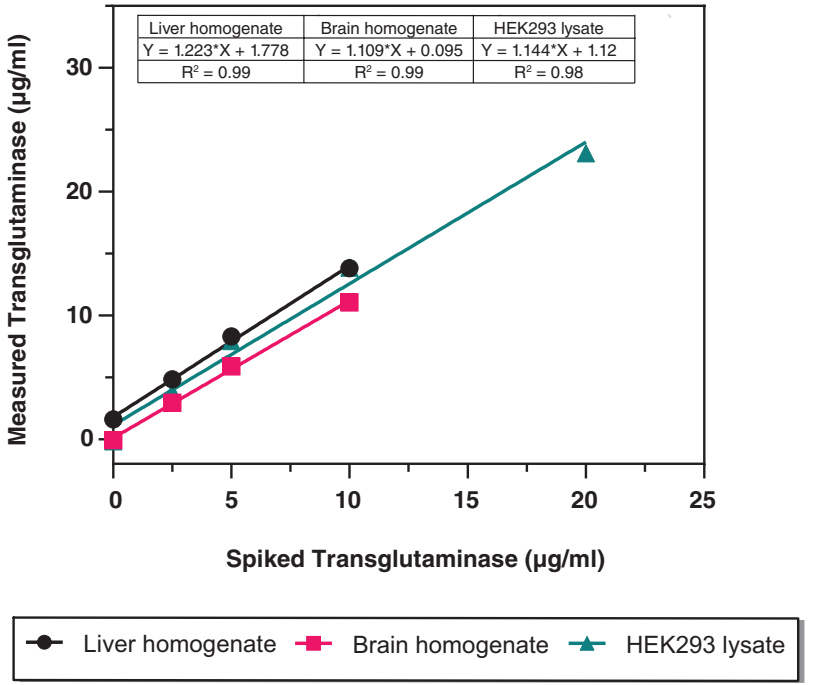


Figure 5. Spike and recovery of transglutaminase in homogenates of rat liver and brain and HEK293 cell lysate

Performance Characteristics

Sensitivity:

The limit of detection (LOD) for this assay is 0.06 U/ml.

Precision:

When a series of 24 blank and positive control measurements were performed on the same day under the same experimental conditions, the intra-assay coefficient of variation was 4% and 5%, respectively.

Z´ Factor:

Z´ factor is a term used to describe the robustness of an assay, which is calculated using the equation below.[5]

$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{|\mu_{c+} - \mu_{c-}|}$$

Where σ: Standard deviation
μ: Mean
c+: Positive control
c-: Negative control

The theoretical upper limit for the Z' factor is 1.0. A robust assay has a Z' factor >0.5. The Z' factor for Cayman's Transglutaminase Activity Assay Kit was determined to be 0.84.

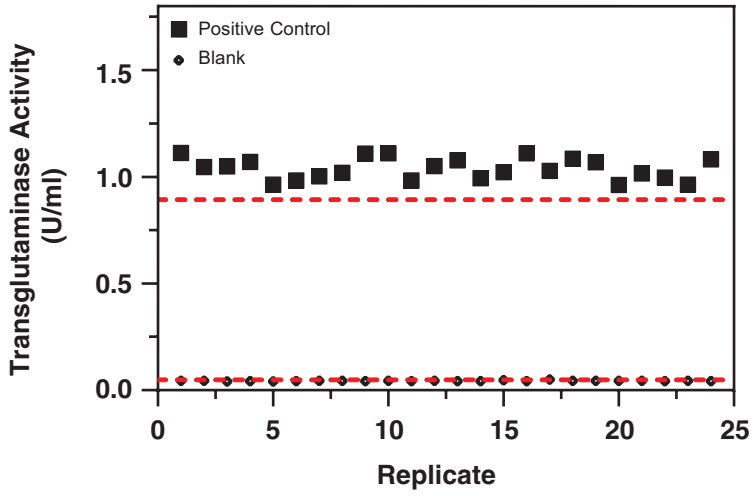


Figure 6. Typical performance data for the Transglutaminase Activity Assay Kit. Data are shown from 24 replicates each for the positive control and blank wells prepared as described in the kit booklet. The calculated Z' factor for this experiment was 0.84. The red lines correspond to three standard deviations from the mean for each control value.

Interferences

Any reagent at a concentration that will denature enzymes will interfere with the assay. In addition, the following reagents were tested for interference in the assay.

Reagent		Will Interfere (Yes or No)
Buffers	M-PER™	No
	T-PER™	No
	100 mM HEPES, pH 7.6, 40 mM $CaCl_2$, and 10 mM DTT	No
	RIPA Buffer	Yes
Chelators	EDTA and other iron chelators	Yes
Protease Inhibitors	EDTA-free Protease Inhibitor Cocktail	No

Table 2. Interferences

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No increase in absorbance when compared to blank or sample background wells	A. Sample was not added to the wells B. Transglutaminase activity is too low to detect	A. Make sure to add all the components to the well(s) and re-assay B. Prepare a more concentrated tissue homogenate or cell lysate
High sample background (A_{525} >0.3)	A. Sample is hemolytic B. Protein concentration in sample is too high	A. Rinse tissues well with PBS prior to homogenization B. Dilute samples with homogenate/lysis buffer and re-assay

References

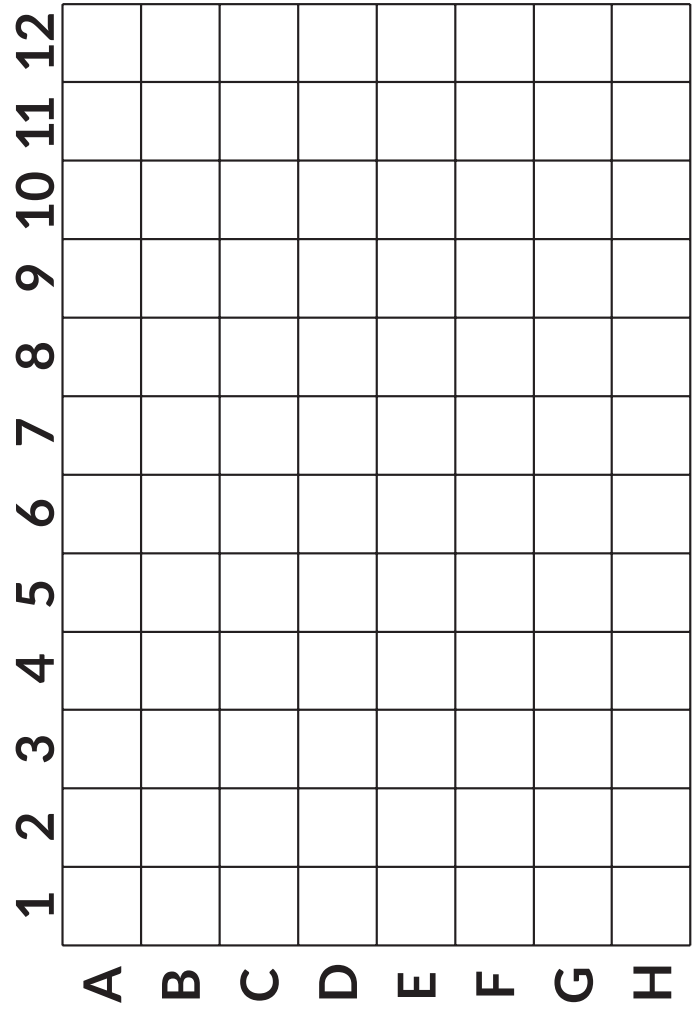
1. Ricotta, M., Iannuzzi, M., De Vivo, G., *et al.* Physio-pathological roles of transglutaminase-catalyzed reactions. *World J. Biol. Chem.* **1(5)**, 181-187 (2010).
2. Klöck, C. and Khosla, C. Regulation of the activities of the mammalian transglutaminase family of enzymes. *Protein Sci.* **21(12)**, 1781-1791 (2012).
3. Griffin, M., Casadio, R., and Bergamini, C.M. Transglutaminases: Nature's biological glues. *Biochem. J.* **368(Pt 2)**, 377-396 (2002).
4. Odii, B.O. and Coussons, P. Biological functionalities of transglutaminase 2 and the possibility of its compensation by other members of the transglutaminase family. *ScientificWorldJournal* **714561** (2014).
5. Zhang, J.H., Chung, T.D.Y., and Oldenburg, K.R. A simple statistical parameter for use in evaluation and validation of high throughput screening assay. *J. Biomol. Screen.* **4(2)**, 67-73 (1999).

Reagent	Procedure
Transglutaminase Assay Substrate 1	Reconsitute each vial with 2 ml of pure water
Transglutaminase Assay Substrate 2	Dilute 1:10 with pure water
Transglutaminase Assay Stop Solution	This is ready to use as supplied
Transglutaminase Assay Positive Control	This is ready to use as supplied

Table 3. Reagent preparation summary

	Blank Wells	Positive Control Wells	Sample Wells	Sample Background Wells
Reconstituted Transglutaminase Assay Substrate 1	40 µl	40 µl	40 µl	40 µl
Homogenate/Lysis Buffer	10 µl	--	--	10 µl
Transglutaminase Assay Positive Control	--	10 µl	--	--
Sample	--	--	10 µl	10 µl
Cover the plate and incubate at 37°C for five minutes				
Diluted Transglutaminase Assay Substrate 2	10 µl	10 µl	10 µl	--
Cover the plate and incubate at 37°C for 20 minutes				
Transglutaminase Assay Stop Solution	60 µl	60 µl	60 µl	60 µl
Read absorbance at 525 nm				

Table 4. Assay summary



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.