SensoLyte® Transglutaminase Activity Assay Kit
Colorimetric

Revision number: 1.0 Last updated: 4/12/2016

Catalog #	AS-72244
Kit Size	100 assays (96-well plate)

- ***Optimized Performance:*** Optimized to detect activity of human transglutaminase enzyme.
- ***Enhanced Value:*** Ample reagents to perform 100 assays in a 96-well format.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	96 well plate, coated with Poly-D-Lysine, includes adhesive sealing film	1 plate
Component B	Wash/Assay buffer (10X)	25 mL
Component C	Biotin-peptide substrate	50 µL
Component D	Human transglutaminase-2, recombinant	2.5µg, 40 µL
Component E	Transglutaminase reaction buffer	15 mL
Component F	DTT, 1M	150 µL
Component G	Streptavidin HRP conjugate	25 µL
Component H	TMB substrate	10 mL
Component I	Stop solution	10 mL
Component J	Transglutaminase inhibitor	1mM, 25 µL

Other Materials Required (but not provided)

- Colorimetric microplate reader: Capable of detecting absorbance at 450nm.

Storage and Handling

- Store component D at -80°C
- Store components C, F, G, and J at -20°C
- Store components A, B, E, H, and I at 4°C
- Protect components G, H from light

Introduction

Transglutaminases (TGs) are Ca^{2+} and thiol-dependent enzymes that catalyze posttranslational modifications of proteins. These covalent reactions include formation of isopeptide linkages between the carboxamide groups of protein-bound glutamine residues and ε-amino groups of protein-bound lysine and polyamine residues, hydrolysis and esterification of glutamine residues .[1] Family of TGs includes at least nine members: TG 1-7, coagulation factor XIIIa, and the keratinocyte membrane bound TG form.[1-2] TG has attracted considerable interest as a potential drug target. Its activity is thought to contribute to Alzheimer disease, Parkinson disease, Huntington disease, and supranuclear palsy.[3-5]

SensoLyte® Transglutaminase Activity Assay Kit provides a convenient assay for the high throughput screening of TG modulators and inhibitors. TG catalyzes covalent bond formation between a free amine group of poly-D-lysine, which is coated on the plate surface, and γ-carboxamide group of glutamine in biotin-TVQQEL peptide substrate. The reaction immobilizes biotin-conjugated peptide to the plate surface. The amount of immobilized biotin is determined using streptavidin-horseradish peroxidase (HRP) and TMB substrate. Colorimetric signal generated by HRP and TMB substrate is proportional to the activity of TG in a sample. Assay is performed in a convenient 96-well microplate format.

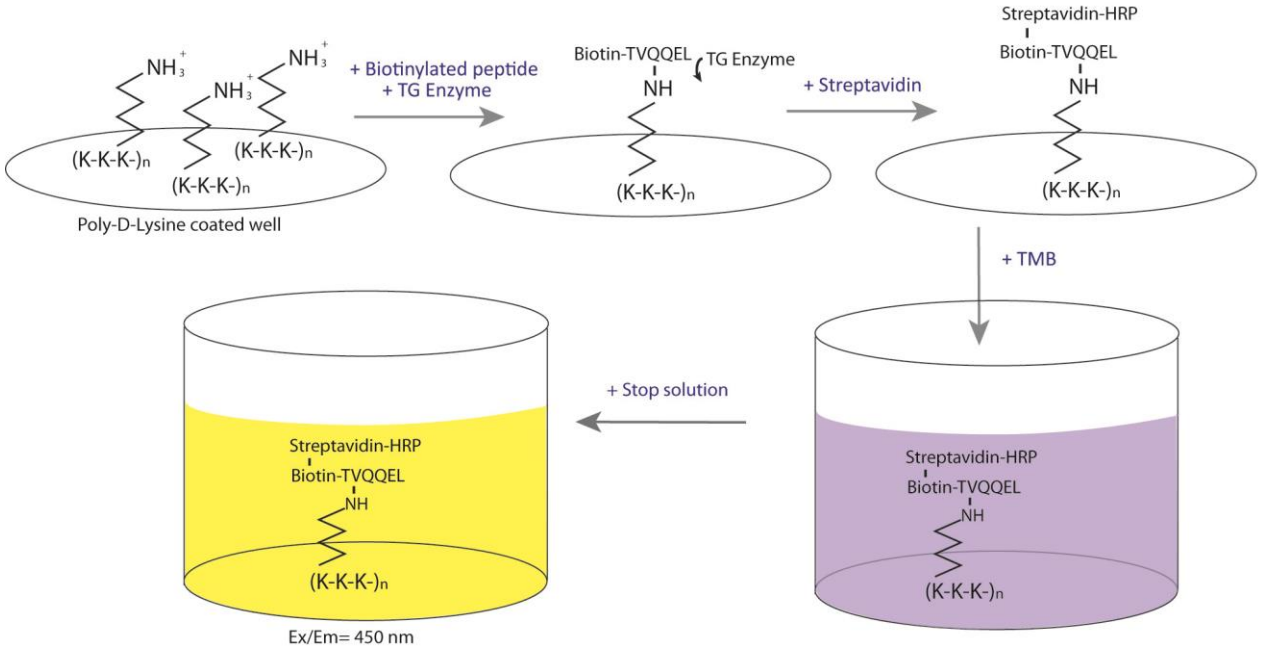

Figure 1. Transglutaminase activity assay principle.
Purified TG or TG containing sample is mixed with Biotin-TVQQEL peptide substrate in the poly-D-lysine coated wells and incubated for 2 h at 37 ºC. Streptavidin-HRP is added to the wells and reacts with cross-linked biotin substrate. Consequently TMB substrate reacts with HRP enzyme and produces colorimetric signal that can be read at 450nm. Color signal is proportional to the TG activity in a sample.

Protocol

Note: Keep transglutaminase enzyme (Component D) on ice.
Warm all other kit components until thawed to room temperature before starting the experiments.

Protocol. Screening of TG modulators/inhibitors using purified or recombinant TG enzyme.

1. Prepare working solutions

1.1 Prepare wash/assay buffer: Dilute 10X Wash/Assay Buffer (Component B) to 1 X with distilled water according to the Table 1. Adjust volume if not using entire plate.

Table 1. Wash/Assay Buffer for one 96-well plate (100 assays)

Components	Volume
10 X Wash/Assay buffer (Component B)	25 mL
Deionized water	225 mL
Total volume	250 mL

1.2 Prepare reaction buffer: Prepare fresh reaction buffer for each experiment according to the Table 2. Adjust volume if not using entire plate. **Use this DTT-containing reaction buffer in all consecutive steps.**

Table 2. Reaction buffer for one 96-well plate (100 assays)

Components	Volume
Transglutaminase reaction buffer (Component E)	9.99 mL
1 M DTT (Component F)	100 µL
Total volume	10 mL

1.3 Biotin-peptide substrate solution: dilute biotin-peptide (Component C) 100-fold with reaction buffer from Step 1.2 according to the Table 3. Each assay will require 50µL of the biotin-peptide substrate. Adjust volume if not using entire plate.

Table 3. Biotin-peptide substrate for one 96-well plate (100 assays).

Components	Volume
Biotin-peptide (Component C)	50 µL
Reaction buffer	4.95 mL
Total volume	5 mL

1.4 Transglutaminase enzyme diluent: dilute enzyme (Component D) 100-fold with reaction buffer from Step 1.2 according to the Table 4. Suggested volume of TG enzyme is 40µl/well. Adjust volume if not using entire plate.

Table 4. TG enzyme solution for one 96-well plate (100 assays).

Components	Volume
TG enzyme (Component D)	40 µL
Reaction buffer	3.96 mL
Total volume	4 mL

Note: Prepare enzyme diluent immediately before use. Do not vortex. Store diluent on ice.

1.5 TG inhibitor solution: dilute ZDON TG inhibitor (Component J) 20-fold with reaction buffer from Step 1.2. Each well will require 10µL of the inhibitor solution.

1.6 Streptavidin-HRP solution: Dilute Streptavidin-HRP conjugate solution (Component G) 500-fold with 1 X Wash/Assay buffer from Step 1.1 according to the Table 5. Prepare Streptavidin-HRP solution before use. Adjust volume if not using entire plate. Each well will require 100µL of the Streptavidin-HRP solution.

Table 5. Streptavidin-HRP solution for one 96-well plate (100 assays).

Components	Volume
Streptavidin-HRP (Component F)	20 µL
1 X Wash/Assay buffer	9.98 mL
Total volume	10 mL

2. Set up enzymatic reaction.

2.1 Add TG enzyme diluent and test compounds into 96-well plate (Component A). Suggested volume for TG enzyme diluent is 40 µL/well and test compound is 10 µL/well.

2.2 Establish the following controls at the same time:

- Positive control contains TG enzyme diluent without test compound.
- Inhibitor control contains TG enzyme diluent and a known TG inhibitor (such as Component J).
- Vehicle control contains TG enzyme diluent and vehicle used to deliver test compound (e.g. DMSO).
- Test compound control contains reaction buffer and test compound.
- Substrate control contains reaction buffer only.

Note: Bring the total volume of all the controls to 50 µL for 96-well plate with reaction buffer.

3. Run assay.

3.1 Add 50 µL of the prepared biotin-peptide substrate solution (from Step 1.3) into each well. Mix reagents completely by gently shaking plate for 30-60 sec.

3.2 Cover wells with adhesive sealing film and incubate plate at 37˚C for 2 h.

3.3 Empty wells and wash 3 times with 200 µL/well of 1 X Wash/Assay buffer (Step 1.1.). Pat dry.

3.4 Add 100 µL/well of prepared Streptavidin-HRP conjugate solution. Incubate plate for 30 minutes at room temp.

3.5 Empty wells and wash 4 times with 200 µL/well of 1 X Wash/Assay buffer (Step 1.1). Pat dry.

3.6 Add 100 µL/well of TMB substrate solution (Component H). Incubate plate for 5-10 minutes at room temp or until vivid blue gradient develops across the wells.

3.7 Add 100 µL/well of Stop solution (Component I).

3.8 Read absorbance at 450nm.

4. Data analysis:

- The absorbance reading from the substrate control well is the background signal. This background reading has to be subtracted from the readings of the other wells.
- For endpoint reading:
  - Plot data as Absorbance versus the concentration of test compounds.
  - A variety of data analyses can be done, e.g., determining inhibition %, EC_{50}, IC_{50}, etc.

References:

1. Ricotta M. et.al., World J Biol Chem 1(5), (2010): 181-187
2. Lesort M. et al., Progr Neurobiol 61, (2010): 439-463
3. Pietsch M. et al., Bioorg Med Chem Let 23, (2013): 6528-6543
4. Verhaar R. et al., Neurochem Intl, (2011): 1-9
5. Hartley D.M. et al, J of Biol Chem 283(24), (2008): 16790-16800