ANASPEC

SensoLyte® Red Transglutaminase Assay Kit *Fluorimetric*

Revision number: 1.1 *Last updated: October 2021*

Catalog #	**AS-72245**
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	ADHP solution	50 µL
Component I	Hydrogen peroxide solution	50 µL
Component J	Transglutaminase inhibitor	1mM, 25 µL

Other Materials Required (but not provided)

- Fluorescence microplate reader: Capable of detecting emission at 590 nm with excitation at 545 nm.

Storage and Handling

- Store component D at -80°C
- Store components C, F, G, H and J at -20°C
- Store components A, B, E, and I at 4°C
- Protect components G , H, and I 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), 10-Acetyl-3,7-dihydroxyphenoxazine (ADHP), and hydrogen peroxide (H_2O_2). The non-fluorescent ADHP is oxidized to the strong fluorescent resorufin in the presence of H_2O_2 by peroxidases, such as HRP. The fluorescence intensity of resorufin is proportional to the activity of TG in a sample. Assay is performed in a convenient 96-well microplate format.

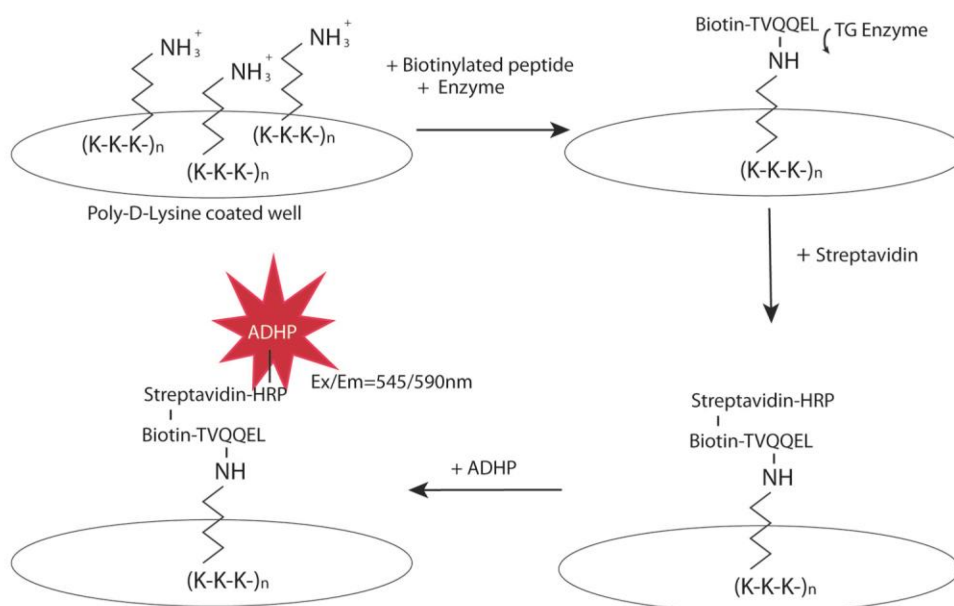
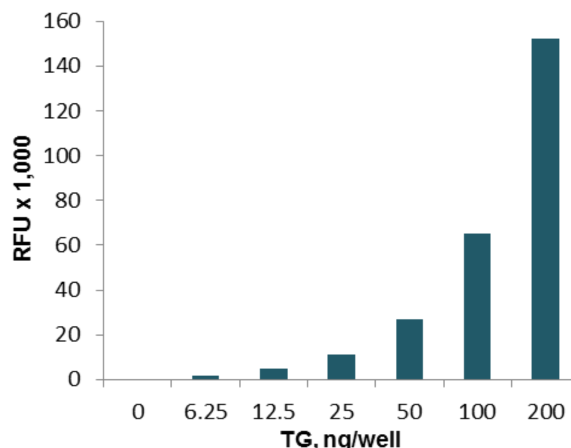


Figure 1. Transglutaminase activity assay principle.
Purified TG or TG containing sample is mixed with Biotin-TVQQEL peptide substrate in the poly-D-lysine coated wells and incubated for 2 h at 37 ºC. Streptavidin-HRP is added to the wells and reacts with cross-linked biotin substrate. Consequently ADHP reacts with HRP enzyme and produces fluorescent signal that can be read at Ex/Em=545/590nm. Fluorescent signal is proportional to the TG activity in a sample.

Figure 2. Detection of TG activity with the SensoLyte® Transglutaminase Activity Assay Kit. Detection limit can reach as low as 120 ng/mL of active human TG enzyme. TG from different sources may vary in its endogenous activity.



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 deionized 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)	9.9 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 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) 100-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 right 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 G)	20 µL
1 X Wash/Assay buffer	9.98 mL
Total volume	10 mL

1.7 ADHP detection solution: Prepare ADHP detection solution according to the Table 6. Prepare ADHP solution right before use. Adjust volume if not using entire plate. Each well will require 100µL of the ADHP detection solution.

Table 6. ADHP detection solution for one 96 well plate (100 assays)

Components	Volume
ADHP (Component H)	50 µL
H_2O_2 (Component I)	10 µL
1 X Wash/Assay buffer	9.94 mL
Total	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 (Component B) 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 the prepared ADHP detection solution. Incubate plate for 15-30 minutes at room temp. Keep plate away from direct light.

3.7 Measure fluorescence intensity at Ex/Em=545nm/590 nm for end-point reading.

4. Data analysis:

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

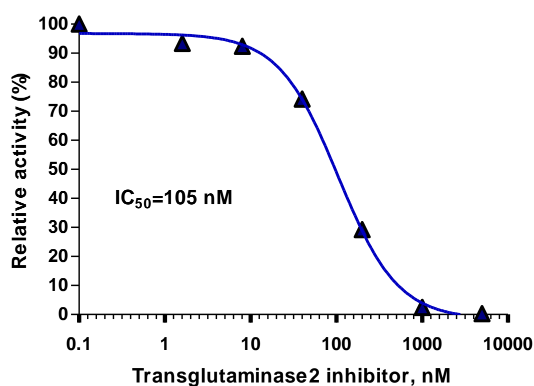


Figure 3. Inhibition of TG activity by Z-DON TG inhibitor as measured with TG activity Assay Kit.

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