



TECHNICAL MANUAL

Transglutaminases (TG) Activity Colorimetric Assay Kit

- **SKU CODE:** MAES0470
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

1. Assay summary

- Reagent preparation
- Standard preparation
- Sample preparation
- Add samples and standards
- Incubate and add chromogenic solution
- Measure absorbance at 525 nm

2. Intended use

This kit can be used to measure transglutaminases (TGs) activity in samples.

3. Detection principle

Transglutaminases (TGs) catalyze the transfer of acyl groups between glutamine residues and lysine residues or primary amines. TG-mediated protein crosslinking is essential for a variety of biological processes and diseases. TGs are also widely used as food processing aids in meat, fish, dairy and grain products.

The detection principle of this kit is: TGs catalyze the acyl transfer between glutamine residues and primary amines. The product forms a chromogenic substance with the chromogenic agent, and TGs activity in the sample is determined by measuring the absorbance change at 525 nm.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	30 mL x 1 vial	-20 °C, 12 months
Reagent 2	Substrate A	Powder x 1 vial	-20 °C, 12 months, protect from light
Reagent 3	Substrate B	1.2 mL x 1 vial	-20 °C, 12 months, protect from light
Reagent 4	Chromogenic Agent A	4 mL x 1 vial	-20 °C, 12 months, protect from light

Item	Component	Size (96 T)	Storage
Reagent 5	Stop Solution	4 mL x 1 vial	-20 °C, 12 months, protect from light
Reagent 6	Chromogenic Agent B	16 mL x 1 vial	-20 °C, 12 months, protect from light
Reagent 7	Standard	Powder x 1 vial	-20 °C, 12 months, protect from light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (520-530 nm, optimum wavelength: 525 nm), Incubator (37 °C)

6. Reagent preparation

1. Equilibrate all reagents to 25 °C before use.
2. **Preparation of substrate A working solution:** Dissolve one vial of substrate A with 3 mL of buffer solution, mix well to dissolve. Keep on ice during use and protect from light. Store at -20 °C for 20 days protected from light.
3. **Preparation of measuring working solution:** For each well, prepare 200 µL of measuring working solution (mix well 160 µL of buffer solution, 20 µL of substrate A working solution and 20 µL of substrate B). The measuring working solution should be prepared fresh and used up within the same day.
4. **Preparation of control working solution:** For each well, prepare 200 µL of control working solution (mix well 180 µL of buffer solution and 20 µL of substrate A working solution). The control working solution should be prepared fresh and used up within the same day.
5. **Preparation of chromogenic working solution:** Before testing, prepare sufficient chromogenic working solution according to the number of test wells. For example, prepare 300 µL of chromogenic working solution (mix well 50 µL of chromogenic agent A, 50 µL of stop solution and 200 µL of chromogenic agent B). The

chromogenic working solution should be prepared fresh and used up within 4 hours.

6. Preparation of 25 mmol/L standard solution: Dissolve one vial of standard with 0.8 mL of buffer solution, mix well to dissolve. The 25 mmol/L standard solution should be prepared fresh, protected from light, and used up within 4 hours.

7. Preparation of standard curve: Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 25 mmol/L standard solution with buffer solution to serial concentrations. The recommended dilution gradient is as follows: 0, 5, 7.5, 10, 12.5, 15, 17.5, 25 mmol/L.

7. Operating steps

1. Standard tube: Add 60 μ L of different concentrations of standard solution to 1.5 mL EP tubes. Sample tube: Add 60 μ L of sample to 1.5 mL EP tubes. Control tube: Add 60 μ L of sample to 1.5 mL EP tubes.
2. Add 200 μ L of measuring working solution to standard and sample tubes. Add 200 μ L of control working solution to control tubes.
3. Centrifuge at 500 x g for 3 min at 4 °C. Incubate at 37 °C for 1 hour protected from light.
4. Add 200 μ L of chromogenic working solution to each tube.
5. Centrifuge at 500 x g for 3 min at 4 °C, then incubate at 37 °C for 10 min protected from light. Centrifuge at 12,000 x g for 10 min at 4 °C.
6. Take 200 μ L of the supernatant from each tube and transfer to the microplate with a micropipette. Measure the OD value of each well at 525 nm with the microplate reader.

8. Calculation

The standard curve:

1. Average the duplicate readings for each standard.
2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.
3. Plot the standard curve using the absolute OD value of standards and corresponding concentration as y-axis and x-axis respectively. Create the standard curve ($y = ax + b$) with graphing software (or Excel).

The sample:

Definition: The amount of enzyme in 1 g protein per 1 hour that produces 1 mmol hydroxamic acid at 37 °C is defined as 1 unit.

$$\text{TGs activity (U/g}_{\text{prot}}) = (\Delta A_{525} - b) \div a \div C_{\text{pr}} \times f \div T$$

[Note]

$$\Delta A_{525}: \Delta A_{525} = OD_{\text{sample}} - OD_{\text{control}}$$

T: Reaction time, 1 hour

C_{pr}: Concentration of protein in sample, g_{prot}/L

f: Dilution factor of sample before testing

9. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	10.00	15.00	22.00
%CV	2.0	2.9	2.8

Inter-assay Precision

Three samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	10.00	15.00	22.00
%CV	5.0	6.1	5.5

Recovery

Three samples of high, middle, and low concentrations were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 102.7%.

Recovery

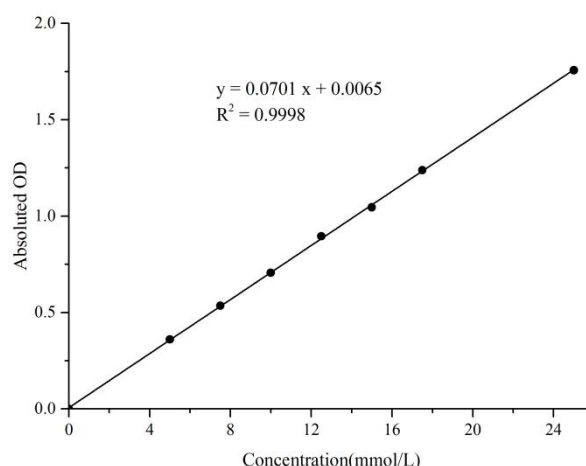
	Standard 1	Standard 2	Standard 3
Expected Conc. (U/L)	10	15	22
Observed Conc. (U/L)	10.0	15.8	22.7
Recovery rate (%)	100	105	103

Sensitivity

The analytical sensitivity of the assay is 3.0 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, or temperature effects), the standard curve and data provided below are for reference only:



Standard curve data

Concentration (mmol/L)	OD value 1	OD value 2	Average OD value	Absolute OD value
0	0.065	0.065	0.065	0
5	0.413	0.438	0.426	0.361
7.5	0.604	0.598	0.601	0.536
10	0.749	0.793	0.771	0.706
12.5	0.961	0.960	0.961	0.896
15	1.122	1.099	1.111	1.046
17.5	1.314	1.294	1.304	1.239
25	1.769	1.876	1.822	1.757

10. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.



Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.