（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K840-M

Specification：96T (40 samples)

Measuring instrument：Microplate reader(520-530 nm)

Detection range：3.0-25.0 U/L

Elabscience® Transglutaminases (TGs) Activity Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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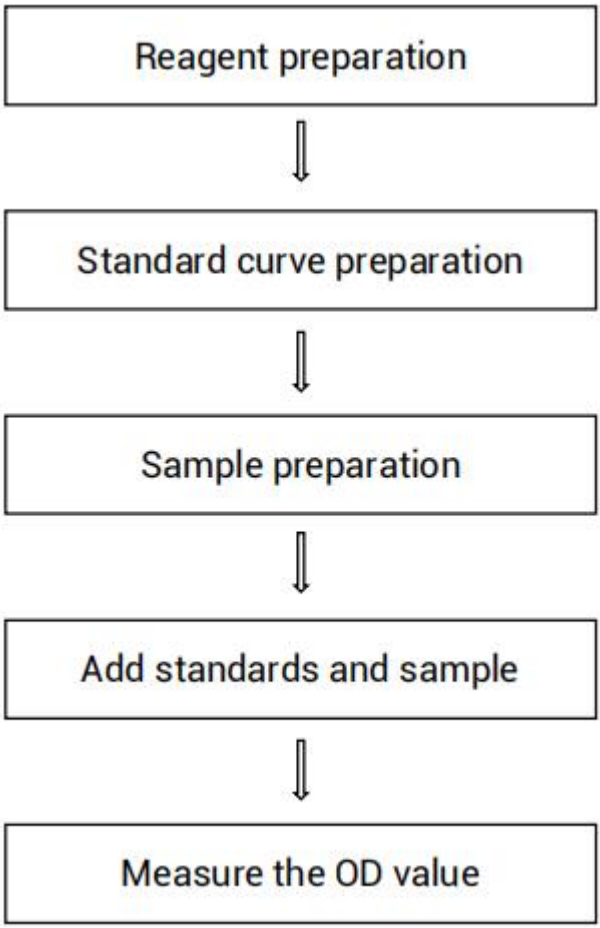
Assay summary



Intended use

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

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 is also widely used as a food processing aid in meat, fish, dairy and grain products.

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

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	30 mL × 1 vial	-20℃, 12 months
Reagent 2	Substrate A	Powder × 1 vial	-20℃, 12 months, shading light
Reagent 3	Substrate B	1.2 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	Chromogenic Agent A	4 mL × 1 vial	-20℃, 12 months, shading light
Reagent 5	Stop Solution	4 mL × 1 vial	-20℃, 12 months, shading light
Reagent 6	Chromogenic Agent B	16 mL × 1 vial	-20℃, 12 months, shading light
Reagent 7	Standard	Powder × 1 vial	-20℃, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:
Microplate reader (520-530 nm, optimum wavelength: 525 nm), Incubator (37℃)

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of substrate A working solution:
Dissolve one vial of substrate A with 3 mL of buffer solution, mix well to dissolve. Keep it on ice during use protected from light. Store at -20°C for 20 days protected from light.

③ The 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 on spot and used up within the same day.

④ The 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 on spot and used up within the same day.

⑤ The preparation of chromogenic working solution:
Before testing, please prepare sufficient chromogenic working solution according to the 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 on spot and used up within 4 h.

⑥ The 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 on spot protected from light and used up within 4 h.

⑦ The 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 a serial concentration. The recommended dilution gradient is as follows: 0, 5, 7.5, 10, 12.5, 15, 17.5, 25 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**5**	**7.5**	**10**	**12.5**	**15**	**17.5**	**25**
25 mmol/L Standard (μL)	0	40	60	80	100	120	140	200
Buffer solution (μL)	200	160	140	120	100	80	60	0

The key points of the assay

During the operation step incubation reaction (③⑤), ensure that the solution on the tube wall slides to the bottom of the tube by centrifugation to reduce the operation error.

Operating steps

① Standard tube: Add 60 μL of different concentrations standard solution to 1.5 mL EP tubes.
Sample tube: Add 60 μL of sample to 1.5 mL EP tubes.
Control tube: Add 60 μL of sample to 1.5 mL EP tubes.

② Add 200 μL of measuring working solution to standard and sample tubes. Add 200 μL of control working solution to control tubes.

③ Centrifuge at 500×g for 3 min at 4℃. Incubated at 37°C for 1 h protected from light.

④ Add 200 μL of chromogenic working solution to each tube.

⑤ Centrifuge at 500×g for 3 min at 4℃, incubated at 37°C for 10 min protected from light. Centrifuge at 12000×g for 10 min at 4℃.

⑥ Take 200 μL the supernatant of each tube to the microplate with a micropipette. Measure the OD value of each well at 525 nm with microplate reader

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

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

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

[Note]

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

T: Reaction time, 1 h.

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

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

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.

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

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 102.7%.

	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

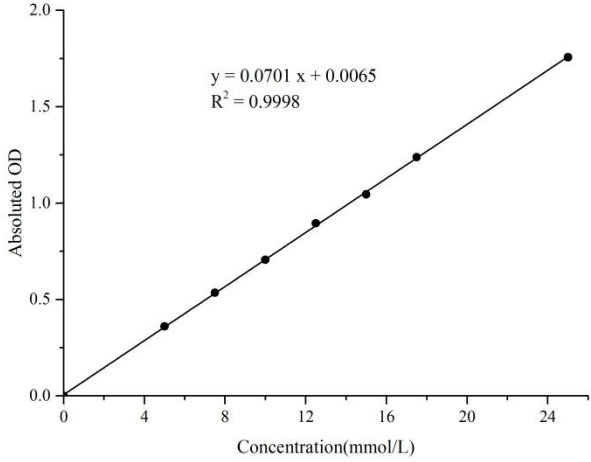
Sensitivity

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.

2. 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), so the standard curve and data are provided as below for reference only：

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



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.