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

Catalog No：E-BC-K1151-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader (445-455 nm)

Detection range：0.29-28.57 U/L

Elabscience® Transketolase(TKT) 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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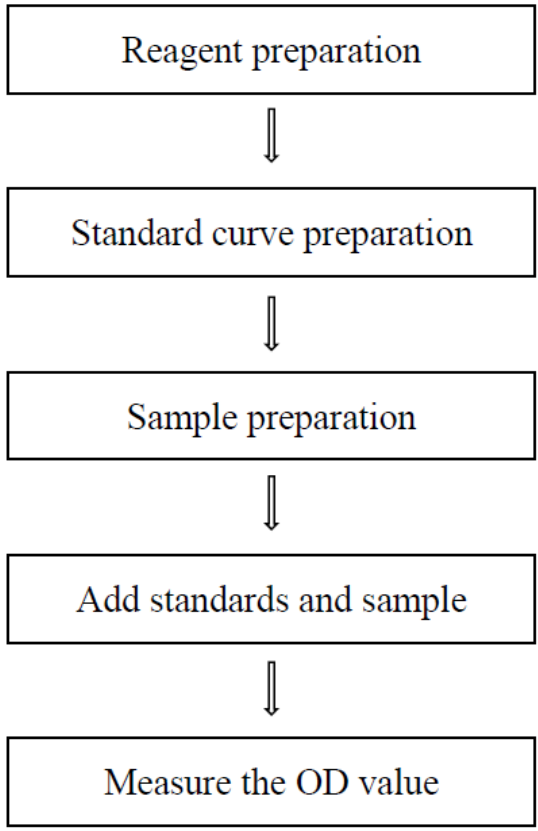
Assay summary



Intended use

The kit is suitable for detecting the activity of Transketolase (TKT) in animal tissues and plant tissues.

Detection principle

Transketolase (TKT) is an important enzyme that catalyzes reactions in the pentose phosphate pathway, participating in the metabolism of various energy substances in the body (such as glucose, ribose, nucleotides, and lipids). TKT can improve blood glucose, glucose tolerance, and β-cell function by inhibiting the formation of advanced glycation end products, thereby reducing oxidative stress, inflammatory responses, atherosclerosis, endothelial dysfunction, and Tau protein phosphorylation, ultimately achieving the prevention and treatment of diabetes and its complications.
TKT catalyzes the reaction of substrates such as xylulose and ribose to generate NADH. NADH reacts with a water-soluble tetrazolium salt to produce the chromogenic formazan, which exhibits a characteristic absorption peak at 450 nm. The activity of TKT is calculated by measuring the rate of increase in absorbance at 450 nm of the sample.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	55 mL × 1 vial	55 mL × 2 vials	-20°C, 12 months, shading light
Reagent 2	Substrate A	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 3	Substrate B	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 4	Coenzyme A	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months, shading light
Reagent 5	Coenzyme B	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months, shading light
Reagent 6	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 7	Chromogenic Agent	3 mL × 1 vial	6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 8	Standard	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Microplate reader (445-455 nm, optimum wavelength: 450 nm), Incubator (37°C)

Materials required but not provided

Double distilled water

Reagent preparation

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

② Preparation of substrate A working solution:
Dissolve one vial of substrate A with 0.4 mL of buffer solution. Stable for 7 d when stored at -20°C protected from light.

③ Preparation of substrate B working solution:
Dissolve one vial of substrate B with 0.4 mL of buffer solution. Stable for 7 d when stored at -20°C protected from light.

④ Preparation of coenzyme A working solution:
Dissolve one vial of coenzyme A with 0.1 mL of buffer solution. Stable for 4 d when stored at -20°C protected from light.

⑤ Preparation of coenzyme B working solution:
Dissolve one vial of coenzyme B with 0.1 mL of buffer solution. Stable for 4 d when stored at -20°C protected from light.

⑥ Preparation of enzyme reagent working solution:
Dissolve one vial of coenzyme with 0.1 mL of buffer solution. Stable for 4 d when stored at -20°C protected from light.

⑦ Preparation of reaction working solution：
For each well, prepare 120 μL of reaction working solution (thoroughly mix 740 μL of buffer solution, 50 μL of substrate A working solution, 40 μL of substrate B working solution, 10 μL of coenzyme A working solution, 100 μL of coenzyme B working solution, 60 μL of enzyme reagent working solution), prepare as needed. The reaction working solution should be freshly prepared before use. Stable for 2 h protected from light.

⑧ Preparation of 0.6 mmol/L Standard Solution：

Dissolve one vial of Standard with 3.33 mL of buffer solution. Aliquot and store unused standard solution at -20°C protected from light for up to 7 days.

Dilute 0.6 mmol/L standard solution with buffer solution diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.12, 0.18, 0.24, 0.3, 0.36, 0.42, 0.6 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.12**	**0.18**	**0.24**	**0.3**	**0.36**	**0.42**	**0.6**
0.6 mmol/L Standard (μL)	0	40	60	80	100	120	140	200
buffer solution (μL)	200	160	140	120	100	80	60	0

Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation
20 mg).

② Wash tissue in cold Buffer Solution.

③ Homogenize 20 mg tissue in 180 μL buffer with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Rat liver tissue homogenization	1-2
10% Mouse liver tissue homogenization	1-2
10% Pig liver tissue homogenization	1-2
10% Mouse Kidney tissue homogenization	1
10% Mouse brain tissue homogenization	1
10% Spinach leaf tissue	1

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 20 μL of standard solution with different concentrations to the corresponding wells.
Sample well: Add 20 μL of sample to the corresponding wells.

② Add 50 μL of Chromogenic Agent to each well.

③ Add 120 μL of reaction working solution to each well.

④ Mix fully with microplate reader for 5 s. Measure the initial OD value (A_1) of each well at 450 nm with microplate reader.

⑤ Incubate at 37°C for 30 min. Measure the OD value (A_2) of each well. (The standard curve is fitted to the standard well in A_2 value).

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 Corrected OD value.

3. Plot the standard curve by using Corrected 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:

Tissue :

Definition: One unit of enzyme is defined as the amount enzyme in 1 g protein will catalyze substrate to produce 1 μmol product per 1 min at 37 °C.

$$\text{CES activity (U/gprot)} = (\Delta A_{sample} - \Delta A_{control} - b) \div a \div T \div \frac{m}{V} \times f \times 1000$$

[Note]

ΔA_{sample}: The OD value of the sample well changes., $A_2 - A_1$.

$\Delta A_{control}$: The OD value of the standard (standard concentration is 0) well changes., $A_2 - A_1$.

T: Reaction time, 30 min.

V: Volume of homogenate added with buffer solution, mL.

f: Dilution factor of sample before test.

m: The wet of sample, 0.1g.

1000: 1 mmol/L = 1000 μmol/L.

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Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5	15	25
%CV	4.8	4.2	3.5

Inter-assay Precision

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

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5	15	25
%CV	7.2	6.8	5.0

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 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	5	15	25
Observed Conc. (U/L)	5.1	15	24.5
Recovery rate (%)	102	100	98

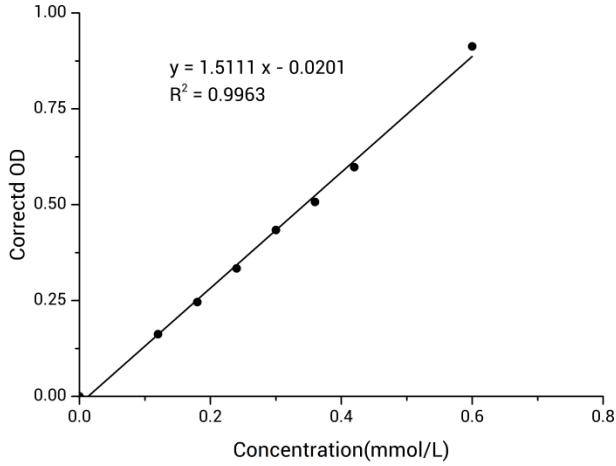
Sensitivity

The analytical sensitivity of the assay is 0.29 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 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	0.12	0.18	0.24	0.3	0.36	0.42	0.6
OD	0.549	0.703	0.797	0.882	0.976	1.053	1.148	1.460
	0.536	0.705	0.780	0.871	0.951	1.045	1.132	1.450
Average OD	0.543	0.704	0.789	0.877	0.976	1.049	1.140	1.455
Corrected OD	0.000	0.162	0.246	0.334	0.434	0.507	0.598	0.913



Appendix II Example Analysis

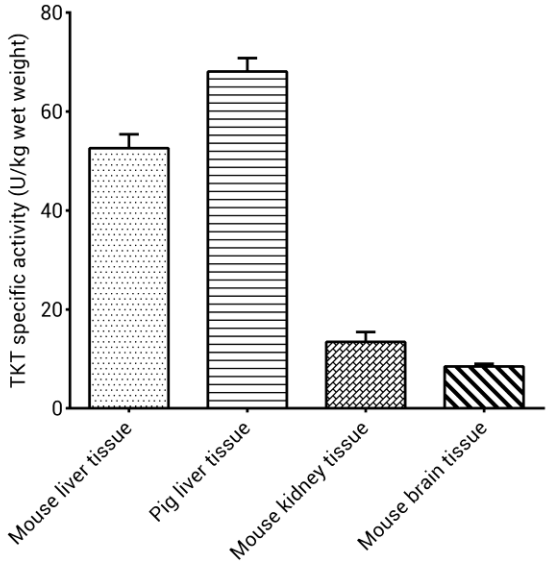
Example analysis：

Take 20 μL of the 2-fold diluted 10% mouse liver tissue homogenate supernatant to the well of microplate. Proceed according to the operating steps. The results are as follows:

standard curve: y = 1.5111 x - 0.0201. The A_1 of control is 0.151, the A_2 of control is 0.543, $\Delta A_{control} = A_2 - A_1 = 0.543 - 0.151 = 0.392$, The A_1 of sample is 0.304, the A_2 of sample is 0.840, $\Delta A_{sample} = A_2 - A_1 = 0.840 - 0.304 = 0.536$, and the calculation result is:

TKT activity (U/kg wet weight) = (0.536 - 0.392 + 0.0201) ÷ 1.5111 ÷ 30 ÷(0.1 ÷ 0.9) × 2 × 1000 = 65.16 U/kg wet weight

Detect 10% mouse liver tissue homogenization (dilute for 2 times), 10% pig liver tissue, 10% mouse kidney tissue and 10% mouse brain tissue, according to the protocol, the result is as follows：



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.