

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

Catalog No: E-BC-K1156-M

Specification: 96T(40 samples)

Measuring instrument: Microplate reader (440-460 nm)

Detection range: 0.001-0.5 mmol/L

Elabscience® Sorbitol 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

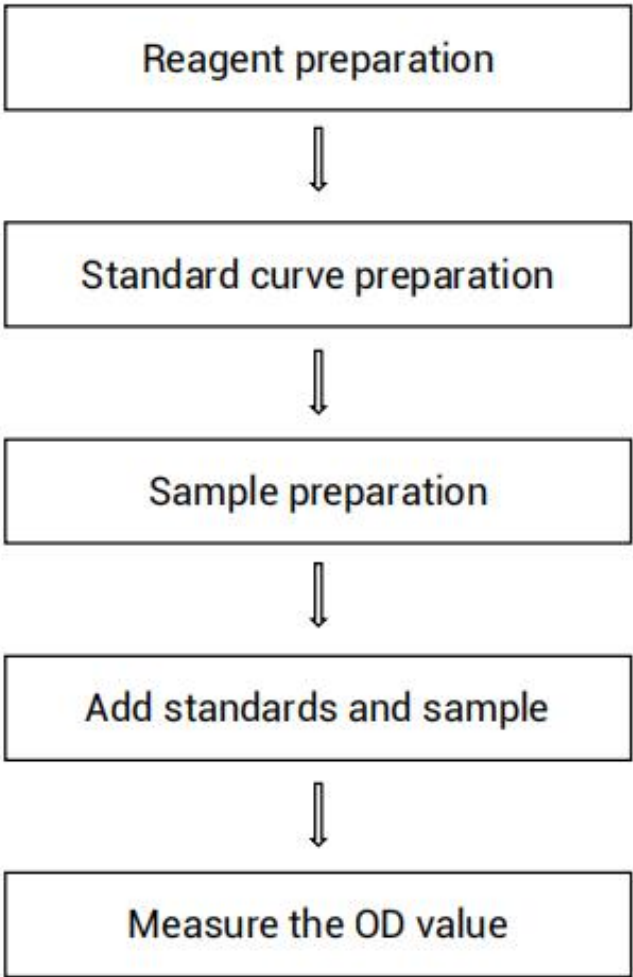
Website: www.elabscience.com

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

Table of contents

Assay summary	3
Intended use	4
Detection principle	4
Kit components & storage	4
Instruments	5
Materials required but not provided	5
Reagent preparation	5
Sample preparation	7
Operating steps	8
Calculation	9
Appendix I Performance Characteristics	10
Appendix II Example Analysis	13
Statement	14

Assay summary



Intended use

The kit is suitable for detecting the activity of acetate kinase (AcK) in tissues and cells samples.

Detection principle

Sorbitol, a six-carbon polyol, is involved in the sorbitol metabolic pathway (also known as the polyol pathway), one of the glucose metabolic pathways in cells. Abnormal activation of the sorbitol metabolic pathway is closely associated with chronic diabetic complications.

Sorbitol dehydrogenase catalyzes the reaction of sorbitol with NAD^+ to generate NADH. NADH reacts with WST-8 to produce the chromogenic formazan, which exhibits a characteristic absorption peak at 450 nm. The sorbitol content is calculated by measuring the absorbance at 450 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	22 mL ×1 vial	-20°C, 12 months, shading light
Reagent 2	Enzyme Reagent	1.1 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Substrate	Powder ×4 vials	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent	11 mL ×1 vial	-20°C, 12 months, shading light
Reagent 5	50 mmol/L Standard	1 mL × 1 vial	-20°C, 12 months, shading light
	Microplate	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 (440-460 nm, optimum wavelength: 450 nm), Incubator (37°C)

Materials required but not provided

Double distilled water, Normal saline (0.9% NaCl), 10 KD ultrafiltration tube (outer tube: 1.5 mL, inner tube: 0.5 mL)

Reagent preparation

- ① Equilibrate all the reagents to 25°C before use. Enzyme Reagent is recommended to be aliquoted and stored at -20°C protected from light. Vortex for 2 s to mix thoroughly before use.
- ② Preparation of substrate solution:
Dissolve one vial of substrate with 200 µL of double distilled water, mix well to dissolve, place on an ice box protected from light for later use. Stable for 3 day when stored at -20°C protected from light.
- ③ Preparation of assay working solution:
For each well, prepare 100 µL of assay working solution (mix well 80 µL of buffer solution, 10 µL of enzyme reagent, 10 µL of substrate solution). Assay working solution stable for 8 h when stored at 25°C protected from light.

④ Preparation of control working solution:

For each well, prepare 100 μL of control working solution (mix well 90 μL of buffer solution and 10 μL of enzyme reagent). Control working solution stable for 8 h when stored at 25°C protected from light.

⑤ Preparation of 0.5 mmol/L Standard Solution:

For each well, prepare 20 μL of standard solution (mix well 10 μL of 50 mmol/L Standard and 990 μL of double distilled water). Aliquot and store unused standard solution at -20°C protected from light for up to 3 days.

Dilute 0.5 mmol/L standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.5 mmol/L.

Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	0	0.10	0.15	0.20	0.25	0.30	0.35	0.50
0.5 mmol/L Standard (μL)	0	40	60	80	100	120	140	200
Double distilled water (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 normal saline (0.9% NaCl).
- ③ Homogenize 20 mg tissue in 180 μ L normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.
- ④ Centrifuge at 12000 $\times$ g for 10 min at 4°C to remove insoluble material. Transfer the supernatant after centrifugation into a 10 KD ultrafiltration tube, centrifuge at 12000 $\times$ g for 10 min, and collect the filtrate from the outer tube for assay. Valid for detection on the same day.

Cell sample

- ① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).
- ② Wash cells with normal saline (0.9% NaCl).
- ③ Homogenize 1×10^6 cells in 200 μ L normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4°C.
- ④ Centrifuge at 12000 $\times$ g for 10 min at 4°C to remove insoluble material. Transfer the supernatant after centrifugation into a 10 KD ultrafiltration tube, centrifuge at 12,000 $\times$ g for 10 min, and collect the filtrate from the outer tube for assay. Valid for detection on the same day.

Dilution of sample

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

Sample type	Dilution factor
10% Mouse liver tissue homogenization	1
10% Mouse kidney tissue homogenization	1
10% Mouse spleen tissue homogenization	1
10% Mouse lung tissue homogenization	1
10% Mouse brain tissue homogenization	1
10% Mouse heart tissue homogenization	1
10% Pothos leaf tissue	1
1×10^6 HL-60 cells	1
1×10^6 RAW264.7 cells	1
1×10^6 A549 cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

- ① Standard well: Add 20 μ L of double distilled water to the corresponding wells.
Sample well: Add 20 μ L of sample to the corresponding wells.
Control well: Add 20 μ L of sample to the corresponding wells.
- ② Add 100 μ L of assay working solution to standard well and sample well.
- ③ Add 100 μ L of control working solution to control well.
- ④ Add 100 μ L of chromogenic agent to each well.
- ⑤ Mix fully. Incubate at 37°C protected from light for 30 min. Measure the initial OD value of each well at 450 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 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:

- ① Formula for Sorbitol Content in Tissue Samples:.

$$\text{Sorbitol content (mmol/kg wet weight)} = (\Delta A - b) \div a \div \frac{m}{v} \times f$$

- ② Formula for Sorbitol Content in Cell Samples:.

$$\text{Sorbitol content (mmol/10}^9\text{)} = (\Delta A - b) \div a \div \frac{n}{v} \times f$$

[Note]

ΔA : OD value of sample well - OD value of control well.

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

f: Dilution factor of sample before test.

m: The wet of sample, 0.1g.

n: The number of cell sample/ 10^6 .

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

Three mouse liver tissue samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.1	0.2	0.35
%CV	5.0	3.1	4.7

Inter-assay Precision

Three Mouse liver tissue homogenization supernatant samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Three mouse liver tissue 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)	0.1	0.2	0.35
%CV	9.4	2.8	7.4

Recovery

Take three Mouse liver tissue homogenization supernatant 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%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	0.1	0.2	0.35
Observed Conc. (U/L)	0.099	0.212	0.361
Recovery rate (%)	99	106	103

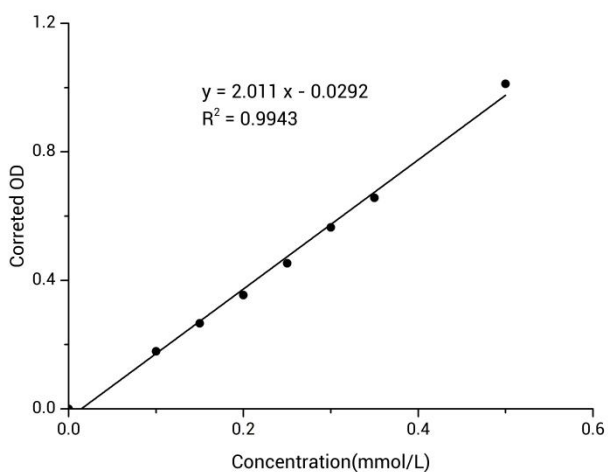
Sensitivity

The analytical sensitivity of the assay is 0.001 mmol/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	0.10	0.15	0.20	0.25	0.30	0.35	0.50
OD	0.269	0.443	0.536	0.613	0.710	0.837	0.915	1.284
	0.268	0.451	0.533	0.631	0.732	0.830	0.936	1.276
Average OD	0.269	0.447	0.535	0.622	0.721	0.834	0.926	1.280
Corrected OD	0.000	0.179	0.266	0.354	0.453	0.565	0.657	1.012



Appendix II Example Analysis

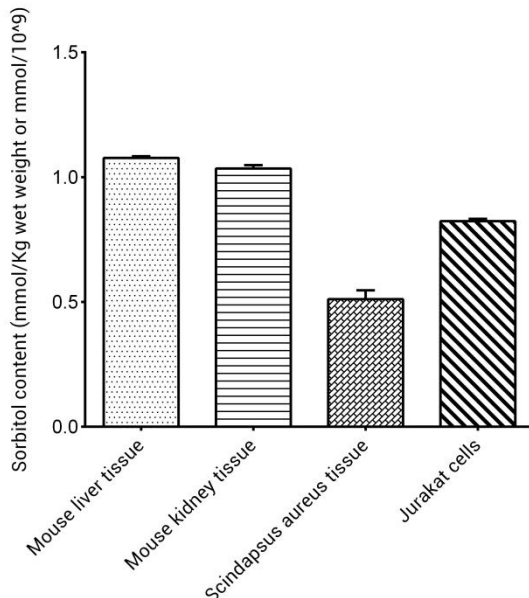
Example analysis :

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

standard curve: $y = 2.011x - 0.0292$. The OD value of sample is 0.348, the OD value of control is 0.146, $\Delta A = 0.348 - 0.146 = 0.202$, and the calculation result is:

$$\begin{aligned}\text{Sorbitol content (mmol/kg wet weight)} &= (0.202 + 0.0292) \div 2.011 \div 0.1 \times 0.9 \\ &= 1.03 \text{ mmol/kg wet weight}\end{aligned}$$

Detect 10% mouse liver tissue homogenization, 10% mouse kidney tissue homogenization, 10% Pothos leaf tissue and 1×10^6 Jurkat cells 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.

