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

Catalog No：E-BC-K1155-M

Specification：48T(24 samples)/96T(48 samples)

Measuring instrument：Microplate reader (440-460 nm)

Detection range：0.59-107.35 U/L

Elabscience® Sorbitol Dehydrogenase(SDH) Activity Colorimetric Assay Kit

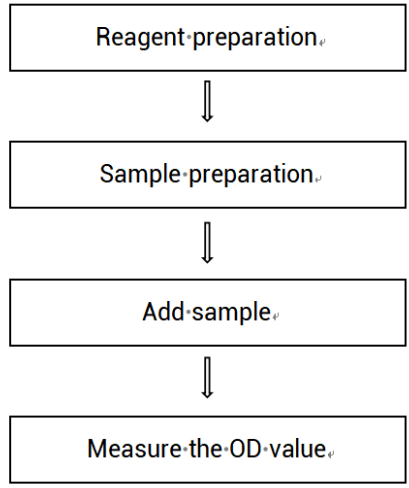
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

The kit is suitable for detecting the activity of sorbitol dehydrogenase (SDH) in tissues, and cells.

Detection principle

Sorbitol dehydrogenase (SDH), a key enzyme in the aldose reductase – sorbitol dehydrogenase metabolic pathway, is closely associated with chronic diabetic complications.

SDH catalyzes the reaction of its sorbitol to produce NADH. The reaction between NADH and the chromogenic reagent exhibits a maximum absorption peak at 450 nm. The enzymatic activity of SDH can be calculated by measuring the increase rate of the OD value at 450 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer	10 mL × 1 vial	20 mL × 1 vial	-20°C, 12 months,
Reagent 2	Cofactor	0.15 mL × 1 vial	0.3 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Substrate A	Powder ×3 vials	Powder ×6 vials	-20°C, 12 months, shading light
Reagent 4	Substrate B	0.3 mL × 1 vial	0.6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Chromogenic Agent	5.5 mL × 1 vial	11 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	Stop Solution	1.2 mL × 1 vial	2.2 mL × 1 vial	-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 (440-460 nm, optimum wavelength: 450 nm), Incubator (37°C)

Materials required but not provided

Double distilled water, 0.9% sodium chloride solution (0.9% NaCl)

Reagent preparation

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

② Preparation of Cofactor Solution：
For each well, prepare 10 μL of Cofactor Solution (mix well 2.5 μL of Cofactor and 7.5 μL of double distilled water). Cofactor Solution should be prepared on spot protected from light and used up within 1 day.

③ Preparation of Substrate A Solution：
Dissolve one vial of Substrate A with 200 μL of double distilled water, mix well to dissolve. Stable for 3 day when stored at -20°C protected from light.

④ Preparation of Color Development Working Solution：

For each well, prepare 100 μL of Color Development Working Solution (thoroughly mix 70 μL of Buffer, 10 μL of Cofactor Solution, 10 μL of Substrate A Solution and 10 μL of Substrate B). Color Development Working Solution should be freshly prepared before use. Stable for 8 h when stored at -20°C protected from light.

⑤ Preparation of Control Working Solution：

For each well, prepare 100 μL of Control Working Solution (thoroughly mix 80 μL of Buffer, 10 μL of Cofactor Solution and 10 μL of Substrate A Solution). Control Working Solution should be freshly prepared before use. Stable for 8 h when stored at -20°C protected from light.

Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).
② Wash tissue in cold 0.9% sodium chloride solution.
③ Homogenize 20 mg tissue in 180 μL 0.9% sodium chloride solution (0.9% NaCl) 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.
⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M/E-BC-K168-M).

Cell sample

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).
② Wash cells with 0.9% sodium chloride solution.
③ Homogenize 1×10^6 cells in 200 μL 0.9% sodium chloride solution (0.9% NaCl) with a ultrasonic cell disruptor 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.
⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M/E-BC-K168-M).

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	2-3
10% Mouse kidney tissue homogenization	1-2
10% Mouse spleen tissue homogenization	1
10% Mouse lung tissue homogenization	1
10% Mouse heart tissue homogenization	1
10% Mouse brain tissue homogenization	1
10% Scindapsus aureus leaf tissue homogenization	1
1×10^6 RAW264.7 cells	1
1×10^6 HL-60 cells	1
1×10^6 A549 cells	1

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

Operating steps

① Sample well: Add 20 μL of sample to the corresponding wells.
Control well: Add 20 μL of sample to the corresponding wells.

② Add 100 μL of Color Development Working Solution to sample well.
Add 100 μL of Control Working Solution to control well.

③ Add 100 μL of Chromogenic Agent to each well.

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

⑤ Incubate at 25°C for 5 min (protected from light).

⑥ Add 20 μL of Stop Solution to each well.

⑦ Mix fully. Measure the OD value (A_2) of each well.

Operating steps

The sample:

Tissue or cell sample:

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.

$$\text{SDH activity (U/gprot)} = (\Delta A_S - \Delta A_C) \times V_1 \times f \div \varepsilon \div d \div V_2 \div T \div C_{pr}$$

$$= (\Delta A_S - \Delta A_C) \times 108 \div C_{pr} \times f$$

[Note]

ΔA_S: The OD value of the sample well changes, A2 - A1.

ΔA_C: The OD value of the control well changes, A2 - A1.

ε: The molar extinction coefficient of at 450 nm, 3.7×10^4 L/mol/cm.

d: Optical path, 0.6 cm.

V_1: The volume of reaction system, 0.24 mL.

V_2: The volume of sample added to the reaction system, 0.02 mL.

C_{pr}: The concentration of protein in sample, gprot/L.

T: Reaction time, 5 min.

f: Dilution factor of sample before test.

108: Simplified value.

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)	7	20	40
%CV	2.5	3.1	3.6

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)	7	20	40
%CV	5.0	5.8	6.2

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 99.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	7	20	40
Observed Conc. (U/L)	6.86	20	40
Recovery rate (%)	98	100	100

Sensitivity

The analytical sensitivity of the assay is 0.59 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.

Appendix II Example Analysis

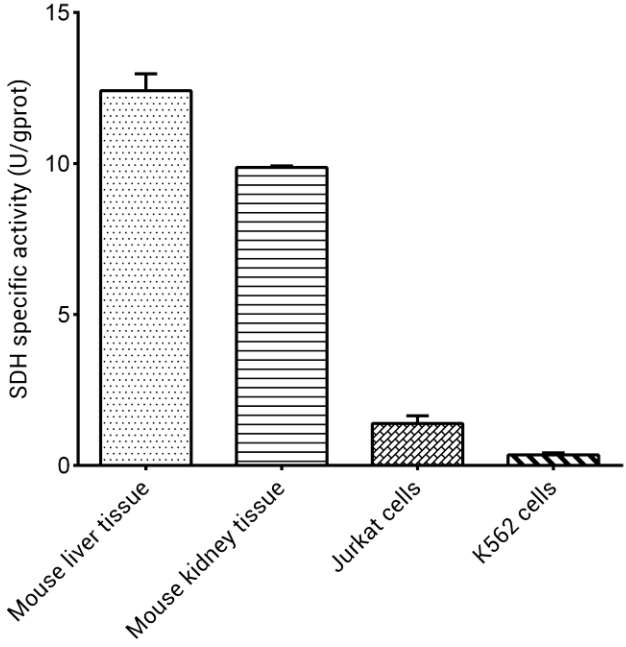
Example analysis：

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:

The concentration of protein is 13.22 gprot/L, and the calculation result is:

SDH activity(U/gport) = (1.000 - 0.242) × 108 ÷ 13.22 × 2 = 12.38 U/gport

Detect 10% mouse liver tissue homogenization (the concentration of protein is 13.22 gprot/L, dilute for 2 times), 10% mouse kidney tissue homogenization (the concentration of protein is 9.40 gprot/L, dilute for 1 times), 1.0×10^6 Jurkat cells (the concentration of protein is 3.80 gprot/L) and 1.0×10^6 K562 cells (the concentration of protein is 4.58 gprot/L), 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.