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

Catalog No：E-BC-K810-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader (430-470 nm)

Detection range：0.05-45.00 U/L

Elabscience®Aldo-Keto Reductase (AKRs) Activity 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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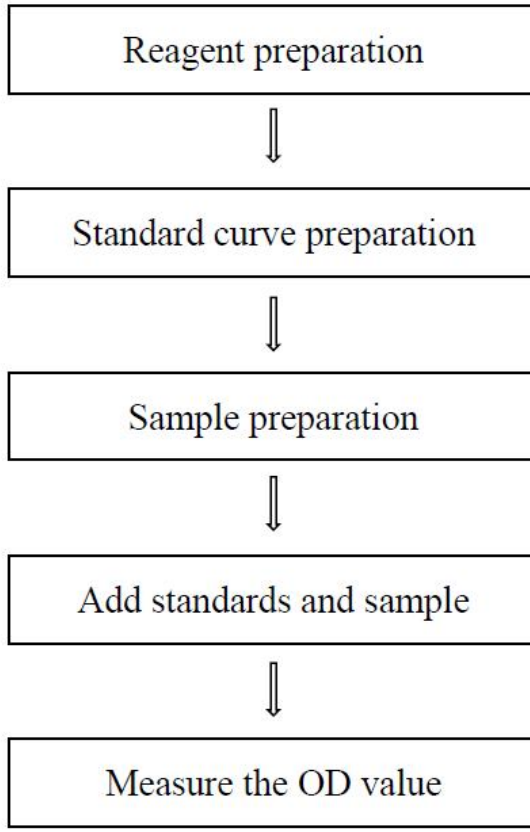
Assay summary



Intended use

This kit can be used to measure aldo-keto reductase (AKRS) activity in serum, plasma and tissue samples.

Detection principle

Aldo-keto reductase (AKRS) is distributed in prokaryotes and eukaryotes, and there are a variety of isoforms. AKRS can catalyze the NADPH-dependent carbonyl reduction reaction to generate the corresponding alcohols. Studies have shown that AKRS are associated with a variety of cancers. The principle of this kit is that the substrate is catalyzed by AKRS, and the chromogenic substance produced by the reaction of the product and the chromogenic agent has a characteristic absorption peak at 450 nm. By detecting the amount of chromogenic substance produced per unit time, the activity of AKRS enzyme in the sample can be calculated.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20℃, 12 months, shading light
Reagent 2	Substrate	0.2 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Standard	Powder × 1 vial	-20℃, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

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 (450 nm), Incubator(37℃)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of chromogenic working solution：

Before testing, please prepare sufficient chromogenic working solution according to the test wells. For example, prepare 4025 μL of chromogenic working solution (mix well 4000 μL of buffer solution and 25 μL of substrate). The chromogenic working solution should be prepared on spot and protected from light. The chromogenic working solution should be used up within 10 min.

③ The preparation of 500 μmol/L standard solution：

Dissolve one vial of standard with 2 mL of double distilled water, mix well to dissolve. The 500 μmol/L standard solution should be prepared on spot and protected from light. The 500 μmol/L standard solution should be used up within 2 hours.

④ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 500 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 100, 200, 250, 300, 350, 400, 500 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**100**	**200**	**250**	**300**	**350**	**400**	**500**
500 μmol/L standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

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

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ 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).

② Dilution of sample

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

Sample type	Dilution factor
10% Mouse kidney tissue homogenization	1
10% Mouse brain tissue homogenization	1
10% Mouse heart tissue homogenization	1
10% Mouse liver tissue homogenization	1
10% Mouse lung tissue homogenization	1
Mouse plasma	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.

The key points of the assay

During the use and preparation of the chromogenic working solution protected from light, and use within 10 min.

Operating steps

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

② Add 180 μL of chromogenic working solution to standard wells and sample wells. Add 180 μL of buffer solution to control wells.

③ Incubate at 37°C for 30 min protected from light and measure the OD value of each well at 450 nm.

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:

1. Serum (plasma) sample:

Definition: The amount of enzyme of in 1 L serum or plasma sample that hydrolyze the substrate to produce 1 μmol NADPH in 1 min at 37°C is defined as 1 unit.

$$\text{AKRS activity (U/L)} = (\Delta A - b) \div a \div t \times f \div 1000$$

2. Tissue sample:

Definition: The amount of enzyme of in 1 g tissue protein that hydrolyze the substrate to produce 1 μmol NADPH in 1 min at 37°C is defined as 1 unit.

$$\text{AKRS activity (U/gprot)} = (\Delta A - b) \div a \div t \div C_{pr} \times f \div 1000$$

[Note]

ΔA: $OD_{Sample} - OD_{Control}$.

t: Reaction time, 30 min.

f: Dilution factor of sample before test.

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

1000：1 mmol/L = 1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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)	5.00	10.00	12.00
%CV	1.9	2.3	4.8

Inter-assay Precision

Three human serum 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)	5.00	10.00	12.00
%CV	7.1	8.1	10.0

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. (μmol/L)	150	270	380
Observed Conc. (μmol/L)	28.8	81.6	126
Recovery rate (%)	94	102	105

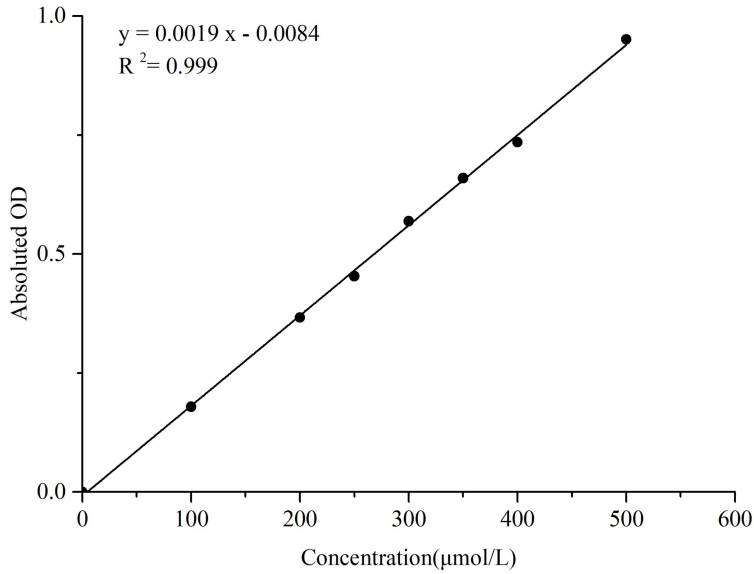
Sensitivity

The analytical sensitivity of the assay is 0.05 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 (μmol/L)	**0**	**100**	**200**	**250**	**300**	**350**	**400**	**500**
OD value	0.054	0.234	0.422	0.51	0.635	0.688	0.795	1.016
	0.053	0.231	0.418	0.504	0.61	0.738	0.782	0.993
Average OD	0.054	0.233	0.420	0.507	0.623	0.713	0.789	1.005
Absoluted OD	0	0.179	0.367	0.454	0.569	0.660	0.735	0.951



Appendix II Example Analysis

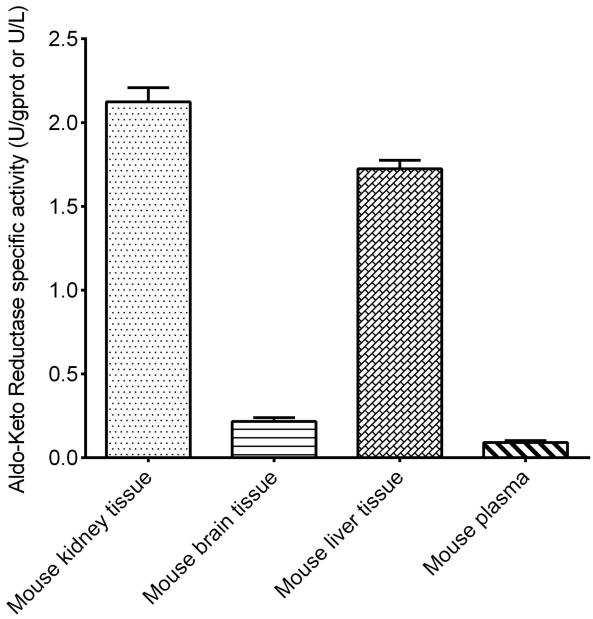
Example analysis：

Take 20 μL of 10% mouse kidney tissue homogenate and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0014 x - 0.0154, the average OD value of the sample is 0.885, the average OD value of the control is 0.248, ΔA = 0.885 - 0.248 = 0.637, the concentration of protein in sample is 7.32 gprot/L, and the calculation result is:

AKRS activity (U/gprot) = (0.885 - 0.248 + 0.0154) ÷ 0.0014 ÷ 30 ÷ 7.32 = 2.122 U/gprot

Detect 10% mouse kidney tissue homogenate (the concentration of protein in sample is 7.32 gprot/L), 10% mouse brain tissue homogenate (the concentration of protein in sample is 4.29 gprot/L), 10% mouse liver tissue homogenate (the concentration of protein in sample is 7.32 gprot/L) and mouse plasma 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.