

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

Catalog No: E-BC-K910-M

Specification: 48T(46 samples)/96T(94 samples)

Measuring instrument: Microplate reader (340 nm)

Detection range: 0.50-125.00 U/L

Elabscience® Acetate Kinase (Ack) 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

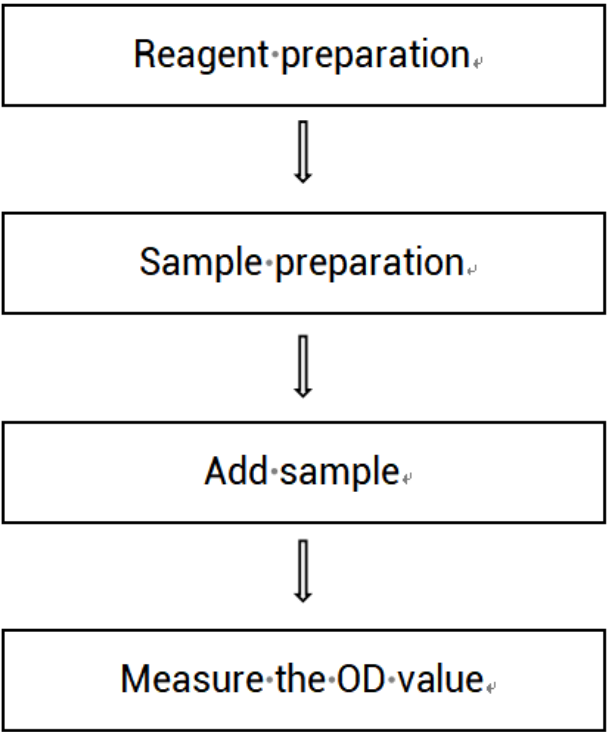
Website: www.elabscience.com

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 acetate kinase (Ack) in serum, plasma, tissues, and cells.

Detection principle

Acetate kinase (Ack) is a member of the ASKHA (acetate and sugar kinases, Hsp70, actin) phosphotransferase superfamily and serves as a central enzyme in carbon and energy metabolism in prokaryotes.

Ack catalyzes the acetate reaction with concomitant consumption of NADH. NADH exhibits a maximum absorption peak at 340 nm, and Ack activity is calculated by measuring the rate of decrease in absorbance at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	13 mL × 1 vial	25 mL × 1 vial	-20°C, 12 months, shading light
Reagent 2	Substrate A	0.9 mL × 1 vial	1.8 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Substrate B	Powder ×3 vials	Powder ×6 vials	-20°C, 12 months, shading light
Reagent 4	Matrix Solution A	Powder ×4 vials	Powder ×8 vials	-20°C, 12 months, shading light
Reagent 5	Matrix Solution B	0.9 mL × 1 vial	1.8 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	Enzyme Reagent	Powder ×1 vial	Powder ×2 vials	-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 (optimum wavelength: 340 nm), Incubator (37°C)

Materials required but not provided

Double distilled water, 1×PBS (0.01 M PBS, pH 7.4)

Reagent preparation

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

② Preparation of substrate A working solution :

For each well, prepare 18 µL of substrate A working solution (mix well 10 µL of substrate A and 90 µL of buffer solution). Substrate A working solution should be freshly prepared before use. Stable for 8 h when stored at 25°C protected from light.

③ Preparation of substrate B working solution :

Dissolve one vial of Substrate B 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 matrix solution A working solution :

Dissolve one vial of matrix solution A with 2000 µL of double distilled water, mix well to dissolve. Stable for 2 day when stored at -20°C protected from light.

⑤ Preparation of matrix solution B working solution :

For each well, prepare 18 µL of matrix solution B working solution (mix well 10 µL of matrix solution B and 10 µL of buffer solution). The matrix solution B working solution stable for 8 h when stored at 25°C protected from light.

⑥ Preparation of enzyme reagent working solution :

Dissolve one vial of enzyme reagent with 200 µL of buffer solution, mix well to dissolve. Stable for 10 day when stored at -20°C protected from light.

For each well, prepare 18 µL of enzyme reagent working solution (mix well 10 µL of enzyme reagent and 40 µL of buffer solution). The enzyme reagent working solution stable for 8 h when stored at 25°C protected from light.

⑦ Preparation of working solution:

For each well, prepare 180 μL of working solution (thoroughly mix 110 μL of Buffer, 20 μL of substrate A working solution, 10 μL of substrate B working solution, 20 μL of matrix solution A working solution, 20 μL of matrix solution B working solution and 20 μL of enzyme reagent working solution). Working solution stable for 4 h when stored at 25°C protected from light

Sample preparation

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

Tissue sample:

- ① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).
- ② Wash tissue in cold 1×PBS (0.01 M PBS, pH 7.4) .
- ③ Homogenize 20 mg tissue in 180 μL 1×PBS (0.01 M PBS, pH 7.4) with a dounce homogenizer at 4°C.
- ④ Centrifuge at 15000×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 1 × PBS (0.01 M PBS, pH 7.4) .
- ③ Homogenize 1×10^6 cells in 200 μL 1 × PBS (0.01 M PBS, pH 7.4) with a ultrasonic cell disruptor at 4°C.

- ④ Centrifuge at 15000×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	2-3
10% Mouse spleen tissue homogenization	1
10% Mouse lung tissue homogenization	1
10% Pig liver tissue homogenization	1
1×10 ⁶ K562 cells	1
1×10 ⁶ Molt-4 cells	1
1×10 ⁶ H9c2 cells	1
1×10 ⁶ ↑ A549 cells	1

Note: The diluent is 1×PBS (0.01 M PBS, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

- ① Control well: Add 20 μL of double distilled water to the corresponding wells.

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

- ② Add 180 μL of Working Solution to each well.
- ③ Mix fully. Incubate at 37°C protected from light for 2 min. Measure the initial OD value (A_1) of each well at 340 nm with microplate reader.
- ④ Incubate at 37°C protected from light for 8 min.
- ⑤ Mix fully. Measure the OD value (A_2) of each well.

Calculation

The sample:

Serum and plasma sample:

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

$$\begin{aligned}\text{AcK activity} \\ (\text{U/L}) &= (\Delta A_S - \Delta A_C) \times V_1 \times f \div \epsilon \div d \div V_2 \div T \div C_{pr} \times 10^6 \\ &= (\Delta A_S - \Delta A_C) \times 334.94 \div C_{pr} \times f\end{aligned}$$

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.

$$\begin{aligned}\text{AcK activity} \\ (\text{U/gprot}) &= (\Delta A_S - \Delta A_C) \times V_1 \times f \div \epsilon \div d \div V_2 \div T \div C_{pr} \times 10^6 \\ &= (\Delta A_S - \Delta A_C) \times 334.94 \div C_{pr} \times f\end{aligned}$$

[Note]

ΔA_S : The OD value of the sample well changes., $A_1 - A_2$.

ΔA_C : The OD value of the control well changes., $A_1 - A_2$.

ϵ : The molar extinction coefficient of at 450 nm., $6.22 \times 10^3 \text{ L/mol/cm}$.

d : Optical path, 0.6 cm.

V_1 : The volume of reaction system, 0.20 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, 8 min.

f : Dilution factor of sample before test.

334.94: Reduced 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)	38	79	111
%CV	2.2	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)	38	79	111
%CV	5.0	6.8	7.2

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 98.5%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	38	79	111
Observed Conc. (U/L)	37.2	78.1	109.7
Recovery rate (%)	98	99	99

Sensitivity

The analytical sensitivity of the assay is 0.50 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

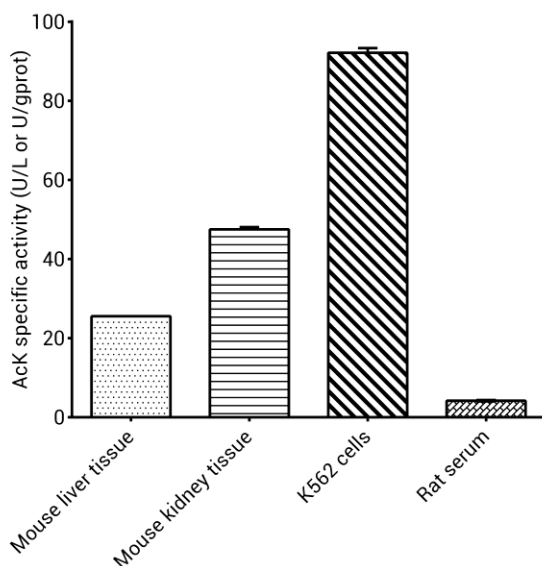
Example analysis:

Take 20 μL of dilute for 3 times 10% mouse liver tissue homogenate supernatant to the well of microplate. Proceed according to the operating steps. The results are as follows:

The A_1 of control is 1.574, the A_2 of sample is 1.425, $\Delta A_{340} = A_2 - A_1 = 1.574 - 1.425 = 0.149$, The A_1 of sample is 1.490, the A_2 of sample is 1.012, $\Delta A_{340} = A_1 - A_2 = 1.490 - 1.012 = 0.478$, the concentration of protein is 13.22 gprot/L, and the calculation result is:

$$\text{AcK activity (U/gprot)} = (0.478 - 0.149) \times 334.94 \div 13.22 = 25 \text{ U/gprot}$$

Detect 10% mouse liver tissue homogenization (the concentration of protein is 13.22 gprot/L, dilute for 3 times), 10% mouse kidney tissue homogenization (the concentration of protein is 9.40 gprot/L, dilute for 3 times), 1.0×10^6 K562 cells (the concentration of protein is 4.58 gprot/L) and rat serum 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.

