PRODUCT INFORMATION & MANUAL

ABP1/AOC1 Activity Assay Kit (Colorimetric)

NBP3-24486

For research use only.
Not for diagnostic or therapeutic procedures.

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ABP1/AOC1 Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24486

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 7.39 U/L

Detection range: 7.39-134.15 U/L

Average intra-assay CV (%): 5

Average inter-assay CV (%): 7

Verage recovery rate (%): 105

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure ABP1/AOC1 activity in serum, plasma, urine, animal tissue and cell samples.

▲Detection principle

ABP1/AOC1 (E.C.1.4.3.6) exists widely in animal tissues (intestinal mucosa, lung, liver, kidney, etc.), serum, plasma and cells. It is a highly active intracellular enzyme in the upper villi of human and mammalian small intestinal mucosa. It can regulate the intracellular ion balance, affect the conduction pathway and promote cell repair. The detection principle of this kit is that ABP1/AOC1 can catalyse amine substances to produce hydrogen peroxide, and hydrogen peroxide can react with the chromogenic substance to produce chromogenic substance, which has a characteristic absorption peak at 460 nm. The activity of ABP1/AOC1 can be calculated by measuring the change rate of absorbance per unit time.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Substrate	1.5 mL × 2 vials	2-8℃ , 12 months, shading light
Reagent 3	Enzyme Reagent	Powder ×1 vial	2-8℃ , 12 months, shading light
Reagent 4	Chromogenic Agent	Powder ×1 vial	2-8℃ , 12 months, shading light
Reagent 5	Stop Agent	10 mL × 1 vial	2-8℃ , 12 months
Reagent 6	100 mmol/L Standard	1 mL × 1 vial	2-8℃ , 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.			

▲Materials prepared by users

Instruments

Centrifuge, Incubator, Microplate reader (450-470 nm, optimum wavelength: 460 nm).

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

⚠ The key points of the assay

1. Prevent the formulation of bubbles when reagent 5 is transferred into the microplate.
2. If the detection system is turbidity after adding reagent 5, mix the reaction system.
3. If there is solid substance in reagent 5, heat it at 60℃ for 5 min until clear.

Pre-assay preparation

▲ Reagent preparation

1. Preserve the reagent 3 on the ice, and bring the rest of the reagents to room temperature before test.
2. Preparation of reagent 3 stocking solution:
   Dissolve reagent 3 with 1.5 mL of double distilled water and mix fully. The prepared solution can be stored at 2-8°C with shading light for 7 days.
3. Preparation of reagent 3 working solution:
   Mix reagent 3 stocking solution with reagent 1 fully at a ratio of 1:9. Prepare the fresh needed amount before use and preserve it on ice with shading light for detection.
4. Preparation of reagent 4 working solution:
   Dissolve reagent 4 with 1.9 mL of double distilled water and mix fully. The prepared solution can be stored at 2-8°C with shading light for 7 days.
5. Preparation of 5 mmol/L standard:
   Mix the reagent 6 and double distilled water at the ratio of 1: 19. Prepare the fresh needed amount before use and the prepared solution can be stored at 2-8°C with shading light for 7 days.

▲ Sample preparation

1. Serum(plasma)sample:

Detect directly. If the sample is turbidity, centrifuge at 2000 g for 10 min, then take the supernatant for detection.

2. Tissue sample:

Accurately weigh the tissue sample, add 9 times the volume of reagent 1 according to the ratio of weight (g): volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant.

3. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (2×10^6): reagent 1 (μL) =1: 200. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (7.39-134.15 U/L).

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

Sample type	Dilution factor
Human serum	2-4
Human plasma	1
Rat serum	1
Rat plasma	1
Mouse serum	1
Mouse plasma	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat heart tissue homogenate	1
Human urine	1
Hela Cells (1×10^6)	1

Note: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, control wells; S1'-S40', sample wells.

▲Detailed operating steps

1. The preparation of standard curve

Dilute 5 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0.0, 0.2, 0.5, 0.7, 1.0, 1.2, 1.5, 2.0 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	5 mmol/L Standard(μL)	Double distilled water (μL)
A	0	0	200
B	0.2	8	192
C	0.5	20	180
D	0.7	28	172
E	1.0	40	160
F	1.2	48	152
G	1.5	60	140
H	2.0	80	120

2. The measurement of samples

(1) Standard well: Take 20 μL of standard solution with different concentrations to the corresponding wells.

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

Control well: Take 20 μL of sample to the corresponding wells.

(2) Add 20 μL of reagent 2 and 100 μL of reagent 3 working solution to each well.

(3) Add 30 μL of double distilled water into the control wells.

Add 30 μL of reagent 4 working solution into the standard wells and sample wells.

(4) Mix fully with microplate reader and incubate at 37℃ for 30 min.

(5) Add 50 μL of reagent 5 into each well (Please take and beat slowly to avoid large bubbles).

(6) Mix fully with microplate reader and incubate at 37℃ for 5 min.

(7) Measure the ABP1/AOC1 value of each well with microplate reader at 460 nm(If there are some bubbles, break it before measurement).

▲Summary operation table

	Standard well	Sample well	Control well
Standards with different concentrations (μL)	20		
Sample (μL)		20	20
Reagent 2 (μL)	20	20	20
Reagent 3 working solution (μL)	100	100	100
Double distilled water (μL)			30
Reagent 4 working solution (μL)	30	30	
Mix fully and incubate at 37℃ for 30 min.			
Regent 5 (μL)	50	50	50
Mix fully and incubate at 37℃ for 5 min and measure the OD value ofwith at 460 nm.			

▲Calculation

Plot the standard curve by using ABP1/AOC1 value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the ABP1/AOC1 value of sample. The standard curve is: y= ax + b.

1. Tissue and cells sample:

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

$$\text{ABP1/AOC1 activity (U/gprot)} = (\Delta A_{460} - b) \div a \div C_{pr} \div T \times f \times 1000^*$$

2. Serum/plasma sample:

Definition: The amount of enzyme in 1 L of serum (plasma) that hydrolysis the substrate to produce 1μmol substance at 37°C for 1 min is defined as 1 unit.

$$\text{ABP1/AOC1 activity (U/L)} = (\Delta A_{460} - b) \div a \div T \times f \times 1000^*$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of Standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{460}: $OD_{Sample} - OD_{Blank}$.

T: The time of incubation reaction, 30 min.

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

f: Dilution factor of sample before test.

1000*: 1 mmol/L =1000 μmol/L

Appendix I Data

▲Example analysis

For human serum, take 20 μL of human serum diluted for 2 times, carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.2474 x - 0.0196, the average ABP1/AOC1 value of the control is 0.058, the average ABP1/AOC1value of the sample is 0.194, and the calculation result is:

DAO activity (U/L) = (0.194 – 0.058 + 0.0196) ÷ 0.2474 ÷ 30 × 2 × 1000

= 41.93 U/L

Appendix II References

1. Aaron P M. The structure and inhibition of human diamine oxidase . Biochemistry. 2009 20: 48(41): 9810–9822.