PRODUCT INFORMATION & MANUAL

Oxaloacetate Assay Kit (Colorimetric)

NBP3-25903

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

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Oxaloacetate Assay Kit
(Colorimetric)

Catalog No: NBP3-25903

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.017 mmol/L

Detection range: 0.017-1 mmol/L

Average intra-assay CV (%): 2.1

Average inter-assay CV (%): 2.3

Average recovery rate (%): 101

- ▲ 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 measure oxaloacetate content in animal tissue sample.

▲Detection principle

Oxaloacetic acid (OAA), one of the intermediates in the tricarboxylic acid cycle is an important substance in carbon and nitrogen metabolism. Oxaloacetate reacts with substrate acetyl-coA under the action of enzyme. The produced substance can react with DTNB, which has the maximum absorption peak at 412 nm. The content of oxaloacetate can be determined by measuring the absorbance value.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20℃ , 12 months
Reagent 2	Substrate	2 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 3	Enzyme Reagent	Liquid × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Chromogenic Agent	2.4 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 5	Standard	Powder × 2 vials	-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.			

▲Materials prepared by users

Instruments

Microplate reader (402-422 nm, optimum wavelength: 412 nm), Incubator, Ultrafiltration tube(50 KD)

Reagents:

Normal saline (0.9% NaCl)

⚠ 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. All reagents should be stored with shading light strictly.
2. Mix reagent 4 fully before use and preserve it on the ice box with shading light during use.
3. Don't dissolve reagent 1 with heating and avoid repeated freeze-thaw.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 2 working solution:
   Mix the reagent 1 and reagent 2 at the ratio of 9:1 fully. The prepared solution can be divided into smaller packages at -20℃ with shading light for 7 days.
3. Preparation of reagent 3 working solution:
   Mix a vial of reagent 3 with 1170 μL of double distilled water fully. Preserve it on the ice box with shading light for use and the prepared solution should be used up within 2 weeks.
4. Preparation of 50 mmol/L standard:
   Dissolve a vial of reagent 5 with 1 mL of double distilled water. Preserve it on the ice box with shading light for use and the prepared solution can be stored at -20℃ with shading light for 7 days.
5. Preparation of 1 mmol/L standard:
   Mix the 50 mmol/L standard and double distilled water at the ratio of 1:49 fully. Prepare the fresh needed amount before use.

▲ Sample preparation

Tissue sample:

Accurately weigh the tissue, add normal saline (0.9% NaCl) at a ratio of Weight (g): Volume (mL) =1:9 and homogenize the sample on ice. Then centrifuge at 12000 g for 15 min, then take the supernatant for centrifugation with a 50 kD ultrafiltration tube at 10000 g for 15 min, and preserve the filtrate on ice for detection.

▲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 (0.017-1 mmol/L).

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

Sample type	Dilution factor
10% Rat liver tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse leg muscle tissue homogenate	1
10% Porcine heart tissue homogenate	1
10% Human urine	1

Note: The diluent is normal saline (0.9% NaCl).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

[Note]: A-H, standard wells; S1, blank wells; S2-S80, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.5, 0.6, 0.8, 1.0 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	1 mmol/L Standard solution (μL)	Double distilled water (μL)
A	0	0	200
B	0.1	20	180
C	0.2	40	160
D	0.3	60	140
E	0.5	100	100
F	0.6	120	80
G	0.8	160	40
H	1.0	200	0

2. The measurement of samples

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

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

(2) Add 140 μL of reagent 2 working solution to each well.

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

(4) Add 20 μL of reagent 4 to each well.

(5) Mix fully with microplate reader for 5 s and incubate at room temperature with shading linght for 3 min. Measure the OD value of each well at 412 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well
Standard solution with different concentrations (μL)	20	
Sample (μL)		20
Reagent 2 working solution (μL)	140	140
Reagent 3 working solution (μL)	20	20
Reagent 4 (μL)	20	20
Mix fully for 5 s and incubate at room temperature for 3 min. Measure the OD value of each well.		

▲Calculation

Plot the standard curve by using OD 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 OD value of sample. The standard curve is: y= ax + b.

For tissue sample:

$$OAA\ (mmol/kg\ wet\ weight) = (\Delta A - b) \div a \div (m \div V) \times f$$

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: $OD_{Sample} - OD_{Blank.}$

m: The weight of the sample, g.

V: The volume of homogenate, mL.

f: Dilution factor of sample before test.

Appendix I Data

▲Example analysis

For 10% Rat liver tissue homogenate, take 20 μL of sample and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.6755 x - 0.007, the average OD value of the control is 0.278, the average OD value of the sample is 0.410, and the calculation result is:

OAA (mmol/kg wet weight) = (0.410 – 0.278 + 0.007) ÷ 0.6755 ÷ (0.1 ÷ 0.9)

= 1.85 mmol/kg wet weight