PRODUCT INFORMATION & MANUAL

Xanthine Oxidase Activity Assay Kit (Colorimetric)

NBP3-24546

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

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

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Xanthine Oxidase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24546

Method: Colorimetric method

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

Instrument: Microplate reader

Sensitivity: 0.067 U/L

Detection range: 0.067-39.30U/L

Average intra-assay CV (%): 3

Average inter-assay CV (%): 9.9

Average recovery rate (%): 99

- 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 xanthine oxidase (XOD) activity in serum, plasma and animal tissue samples.

▲Detection principle

Xanthine Oxidase (XOD) is mainly present in milk, liver and spleen of mammals, belonging to aerobic dehydrogenases, which is an important enzyme in nucleic acid metabolism in the body. When hepatocytes are injured, this enzyme is released into the serum earlier than SGPT, and increase its activity in serum significantly, which has obvious significance for the identification of hepatocellular jaundice and it is used for the identification of obstructive jaundice. In the process of hypoxia, xanthine dehydrogenase quickly forms xanthine oxidase, which plays an important role in free radical production.

XOD can catalyze hypoxanthine to xanthine, and at the same time while producing superoxide anion free radicals. In the presence of electron acceptor and chromogenic agent, the purplish red substance can be generated, and the vitality of XOD can be calculated by measuring the production of purplish red substance.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20°C, 12 months
Reagent 2	Substrate Solution	1 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Enzymatic Reagent	1 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent 1	1.6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Chromogenic Agent 2	1.6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	1 mmol/L Standard Solution	3.2 mL × 1 vial	-20°C, 12 months
	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 (540-560 nm, optimum wavelength: 550 nm), Incubator(37°C)

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.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. The preparation of chromogenic working solution:

   Mix the reagent 4 and reagent 5 at a ratio of 1:1. Prepare the fresh needed amount before use and the prepared solution should be stored with shading light and used up within 1 hour.
3. The preparation of working solution:

   Mix the reagent 1, reagent 2, reagent 3 and chromogenic working solution at a ratio of 147:6.5:6.5:20. Prepare the fresh needed amount before use and the prepared solution should be stored with shading light for use.

▲ Sample preparation

1. Serum (plasma) sample: Detect directly.
2. Tissue sample:

   Weigh the tissue accurately and add normal saline (0.9% NaCl) at a ratio of weight (g): volume (mL) =1: 9, homogenize the tissue in ice bath, centrifuge at 12000 g for 10 min at 4°C, then take the supernatant for measurement. 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 (0.067-39.30 U/L).

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

Sample type	Dilution factor
Rat plasma	1
Rat serum	1
10% Mouse kidney tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Mouse liver tissue homogenate	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-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, 0.2, 0.3, 0.4, 0.6, 0.7, 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	0.2	40	160
C	0.3	60	140
D	0.4	80	120
E	0.6	120	80
F	0.7	140	60
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 into the corresponding wells.

Sample well: Add 20 μL of sample into the corresponding well.

(2) Add 180 μL of working solution into each well.

(3) Mix fully with microplate reader for 5 s, measure the OD values of sample well at 550 nm with microplate reader, recorded as A_1.

(4) Incubation at 37°C for 25 min, measure the OD values of sample well and standard well, recorded as A_2.

▲Summary operation table

	Standard well	Sample well
Different concentrations standard solution (μL)	20	--
Sample (μL)	--	20
working solution (μL)	180	180
Mix fully with microplate reader for 5 s, measure the OD values of sample well at 550 nm with microplate reader, recorded as A_1. Incubation at 37°C for 25 min, measure the OD values of sample well and standard well, recorded as A_2.		

▲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.

1. For serum, plasma sample:

Definition: The amount of xanthine oxidase (XOD) in 1 L serum or plasma sample that hydrolyze the substrate to produce 1 μmol H_2O_2 in 1 minute at 37°C is defined as 1 unit.

$$\text{XOD activity (U/L)} = (\Delta A_{550} - b) \div a \div T \times f \times 1000$$

2. For tissue sample:

Definition: The amount of xanthine oxidase (XOD) in 1 g tissue that hydrolyze the substrate to produce 1 μmol H_2O_2 in 1 minute at 37°C is defined as 1 unit.

$$\text{XOD activity (U/gprot)} = (\Delta A_{550} - b) \div a \div T \times f \div C_{pr} \times 1000$$

Note:

y: $OD_{Standard} - OD_{Blank}$. ($OD_{standard}$ 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_{550}: The change OD of the sample ($\Delta A_{550} = A_2 - A_1$).

T: The time of reaction, 25 min.

f: Dilution factor of sample before tested.

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

1000: 1 mmol/L=1000 μmol/L.

Appendix I Data

▲Example analysis

Take 20 μL of 10% Mouse liver tissue homogenate and carry the assay according to the operation table. The results are as follows: standard curve: y = 0.7121 x + 0.083, the A_1 of the sample is 0.144, incubate at 37°C for 25 min, the A_2 of the sample is 0.453, ΔA_{550} = A_2 – A_1 = 0.453 – 0.144 = 0.309, the concentration of protein in sample is 5.68 gprot/L, and the calculation result is:

XOD activity (U/gprot) = (0.309 - 0.083) ÷ 0.7121 ÷ 25 ÷ 5.68 × 1000 = 2.97 U/gprot