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

Catalog No：E-BC-D019

Specification：96T

Measuring instrument：Microplate Reader (550-565 nm)

Elabscience® Xanthine Oxidase (XOD) Inhibitor Screening 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.

Table of contents

Intended use

This kit is used for the determination of the inhibitory effect of xanthine oxidase (XOD) inhibitors.

Detection principle

Xanthine oxidase (XOD) is an enzyme that catalyzes hypoxanthine hydroxylation to xanthine and xanthine hydroxylation to uric acid, and is excreted by the kidney. Excessive production or insufficient excretion of uric acid can lead to hyperuricemia. Therefore, the development of XOD inhibitors can be used in the treatment of high uric acid and ventilation diseases.

The detection principle of this kit: XOD catalyzed substrate to produce color substances at 560 nm has a maximum absorption wavelength, after adding inhibitors, the generation of color will be inhibited, according to the degree of inhibition to determine the effect of the inhibitor.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 1 vial	-20°C, 12 months
Reagent 2	Substrate	1 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Enzyme Reagent	0.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent	0.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	5 mmol/L Febuxostant	0.2 mL × 1 vial	-20°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

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 (555-565 nm, optimum wavelength: 560 nm)

Reagents:

DMSO

Reagent preparation

① Keep enzyme reagent on ice during use to thawing. Equilibrate other reagents to 25℃ before use.

② The preparation of enzyme working solution：
Before testing, please prepare sufficient enzyme working solution according to the test wells. For example, prepare 200 μL of enzyme working solution (mix well 195 μL of buffer solution and 5 μL of enzyme reagent). Store at 2-8°C for 2 days.

③ The preparation of reaction working solution：
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 505 μL of reaction working solution (mix well 475 μL of buffer solution, 25 μL of substrate and 5 μL of chromogenic agent). The reaction working solution should be prepared on spot and keep it on ice protected from light. The reaction working solution should be used up within 1 day.

④ The use of febuxostant：
The concentration of febuxostat provided in this kit is 5 mmol/L, which

is prepared in DMSO. When febuxostat is used, dilute the febuxostat with buffer solution to the desired concentration. (This reagent is an XOD inhibitor, as a positive control, the determination of inhibition rate can be used as a reference, IC_{50} is about 0.2 μmol/L).

Sample preparation

It is recommended to use buffer solution to dilute the sample. If the sample is poorly water-soluble, DMSO can be prepared into a high-concentration solution and then diluted with buffer solution. The DMSO content in the reaction system should be less than 5%.

The key points of the assay

① The reagent should be prepared protected from light. Keep enzyme reagent on ice during use.

② The positive control well is the inhibition rate of XOD specific inhibitor, which can only be used as a reference. The well can be selected in the actual determination process. The IC_{50} in this kit is about 0.2 μmol/L, and the measured data will be different.

Operating steps

① Blank well: Add 70 μL of buffer solution to the wells;
Total enzyme well: Add 50 μL of enzyme working solution to the wells;
Positive control well: Add 50 μL of enzyme working solution to the wells;
Sample well: Add 50 μL of enzyme working solution to the wells.

② Add 20 μL of buffer solution into total enzyme well. Add 20 μL of febuxostant into positive control well. Add 20 μL of samples into sample well.

③ Mix fully with microplate reader for 3 s and incubate at 25ºC for 5 min protected from light.

④ Add 100 μL of reaction working solution into each well.

③ Mix fully with microplate reader for 3 s and incubate at 25℃ for 2 min protected from light. Measure the OD values of each well at 560 nm with microplate reader.

Calculation

$$\text{Inhibition rate (\%)} = (A_1 - A_2) \div (A_1 - A_3) \times 100\%$$

[Note]

A_1: The OD value of total enzyme well.

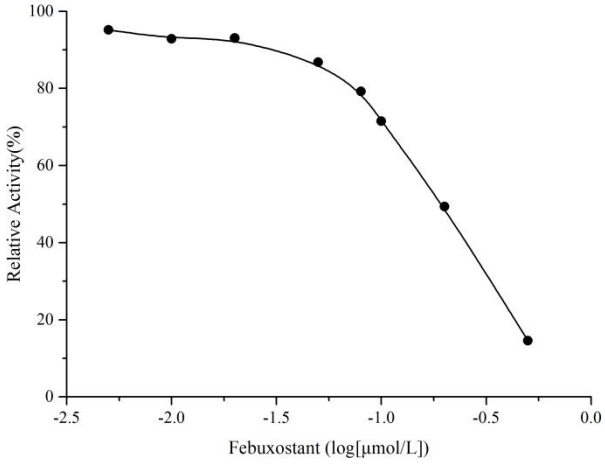
A_2: The OD value of sample well.

A_3: The OD value of blank well.

Appendix I Performance Characteristics

Inhibition curve

The effect of XOD inhibitor screening kit for detection of XOD inhibitor febuxostat inhibitor.



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.