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

Catalog No：E-BC-D002

Specification：96T

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Elabscience® Cyclooxygenase-2 (COX-2) 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
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Intended use

This kit is used for the determination of the inhibitory effect of cyclooxygenase-2 inhibitors.

Detection principle

Cyclooxygenase (COX) is a bifunctional enzyme that exhibits both cyclooxygenase and catalase activities. The cyclooxygenase activity of COX can catalyze the conversion of arachidonic acid (AA) to prostaglandin G2 (PGG2), while the catalase activity of COX can convert prostaglandin G2 to prostaglandin H2 (PGH2).

The detection principle of this kit: Arachidonic acid generates prostaglandins under the catalysis of cyclooxygenase, and under the action of cofactor, cyclooxygenase activates peroxidase activity. After the addition of the inhibitor, the production of fluorescence will be inhibited, and the effect of the inhibitor will be determined according to the degree of inhibition. The fluorescence produced by this kit has a maximum excitation wavelength of 535 nm and a maximum emission wavelength of 587 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20°C, 12 months
Reagent 2	Substrate	0.5 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Diluent	0.5 mL × 1 vial	-20°C, 12 months
Reagent 4	Accelerant	0.1 mL × 1 vial	-20°C, 12 months shading light
Reagent 5	Chromogenic Agent	0.05 mL × 1 vial	-20°C, 12 months shading light
Reagent 6	100 μmol/L Celecoxib	0.5 mL × 1 vial	-20°C, 12 months shading light
Reagent 7	Recombinant COX-2	0.09 mL × 1 vial	-20°C, 12 months shading light
	Black 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator (37℃), Vortex mixer

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of accelerant working solution：

Before testing, please prepare sufficient accelerant working solution according to the test wells. For example, prepare 1255 μL of accelerant working solution (mix well 5 μL of accelerant and 1250 μL of buffer solution). Keep it on ice during use protected from light and used up within 8 hours.

③ The preparation of chromogenic working solution:

Before testing, please prepare sufficient chromogenic working solution according to the test wells. For example, prepare 505 μL of chromogenic working solution (mix well 5 μL of chromogenic agent and 500 μL of double distilled water). The chromogenic working solution should be prepared on spot. Keep it on ice during use protected from light and used up within 4 hours.

④ The preparation of celecoxib working solution:

The positive control inhibitor celecoxib provided with this kit is 100 μM and can be diluted to the desired concentration with double distilled water or buffer solution. Using alcohol reagents and high concentration of surfactant solution as the diluent can produce interference on measurement.

⑤ The preparation of COX-2 working solution:

Before testing, please prepare sufficient COX-2 working solution according to the test wells. For example, prepare 50 μL of COX-2 working solution (mix well 5 μL of recombinant COX-2 and 45 μL of buffer solution). Store at 2-8°C for 3 days protected from light.

⑥ The preparation of substrate working solution:

Solution A: Before testing, please prepare sufficient solution A. For example, prepare 10 μL of solution A (mix well 5 μL of substrate and 5 μL of diluent). Mix fully for 5 s with vortex mixer and stand for 3 min protected from light. The solution A should be prepared on spot. Keep it on ice during use protected

from light and used up within 1 h.

Substrate working solution: Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 55 μL of substrate working solution (mix well 5 μL of solution A and 50 μL of double distilled water). Keep it on ice during use protected from light and used up within 20 min (It is recommended to prepare the substrate working solution during operation of the step ④).

Sample preparation

Take an appropriate amount of the inhibitor to be measured, dilute it with buffer solution or double distilled water to appropriate concentration for use.
Note: For different inhibitors, prepare and dilute with the same solvent.

The key points of the assay

① The substrate working solution deteriorates easily. It is recommended to prepare it in operation of the step ④, and use as soon as possible.
② The setting of positive control wells can determine whether the recombinant protein COX-2 is active.
③ After the sample is dissolved with organic solvent, it is recommended to dilute it with double distilled water or buffer solution to avoid affecting COX-2 enzyme activity.

Operating steps

① Total enzyme well: Add 15 μL of COX-2 working solution to the corresponding wells.
Positive control well: Add 15 μL of COX-2 working solution to the corresponding wells.
Sample well: Add 15 μL of COX-2 working solution to the corresponding wells.
Blank well: Add 15 μL of buffer solution to the corresponding wells.

② Add 70 μL of accelerant working solution into each well.

③ Add 30 μL of inhibitor diluent solution into total enzyme wells and blank wells. Add 30 μL of celecoxib working solution to positive control wells. Add 30 μL of sample to sample wells.

④ Mix fully with fluorescence microplate reader for 3 s and incubate at 37°C for 10 min.

⑤ Add 20 μL of chromogenic working solution into each well.

⑥ Add 50 μL of substrate working solution into each well.

⑦ Mix fully with fluorescence microplate reader for 3 s and incubate at 37°C for 3 min protected from light. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

$$\text{Inhibition Rate (\%)} = (F_{total} - F_{sample}) \div (F_{total} - F_{blank}) \times 100\%$$

[Note]

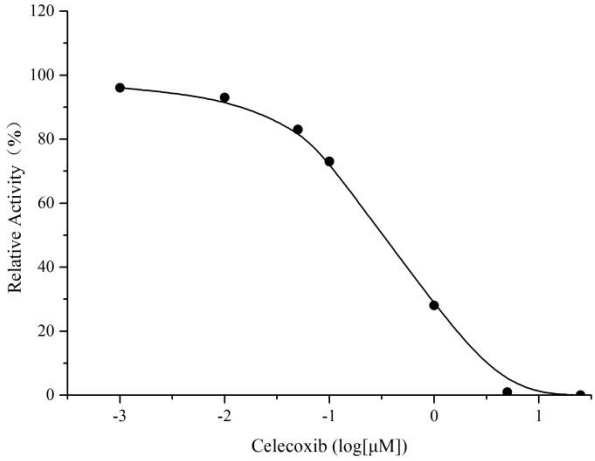
F_{total}: The fluorescence intensity of total enzyme well.

F_{sample}: The fluorescence intensity of sample well.

F_{blank}: The fluorescence intensity of blank well.

Appendix I Performance Characteristics

Celecoxib inhibition rate curve



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.