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

Catalog No：E-BC-F050

Specification：96T

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.03-9.66 U/L

Elabscience®Cyclooxygenase (COX) Activity Fluorometric Assay 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
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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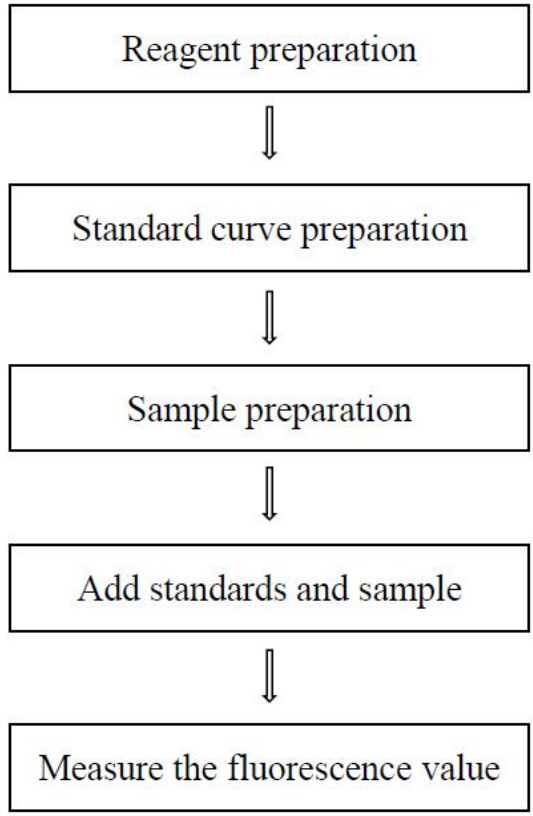
Assay summary



Intended use

This kit can be used to measure COX, COX-1 and COX-2 activity in animal tissue samples.

Detection principle

Cyclooxygenase (COX), also known as prostaglandin endoperoxide synthase (PTGS), 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).

Arachidonic acid generates prostaglandins under the catalysis of cyclooxygenase, and under the action of cofactor (heme), cyclooxygenase activates peroxidase activity, converting fluorescent red into fluorescent substance.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	30 mL × 1 vial	-20℃, 12 months
Reagent 2	Substrate	0.2 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Diluent	0.2 mL ×1 vial	-20℃, 12 months
Reagent 4	Accelerant	0.1 mL × 1 vial	-20℃, 12 months, shading light
Reagent 5	Chromogenic Agent	0.1 mL × 1 vial	-20℃, 12 months, shading light
Reagent 6	2 mmol/L Standard	0.1 mL × 1 vial	-20℃, 12 months, shading light
Reagent 7	1 mmol/L COX-1 Inhibitor	0.3 mL × 1 vial	-20℃, 12 months, shading light
Reagent 8	1 mmol/L COX-2 Inhibitor	0.3 mL × 1 vial	-20℃, 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

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of substrate working solution：

Solution A: For each well, prepare 10 μL of measuring working solution (mix well 5 μL of substrate and 5 μL of diluent). The prepared solution should be prepared on spot 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 250 μL of substrate working solution (mix well 5 μL of solution A and 245 μL of double distilled water). The prepared solution should be prepared on spot and used up within 30 min. (It is recommended to prepare substrate working solution at incubation step.)

③ Preparation of chromogenic working solution：

For each well， add 5 μL of chromogenic agent and 495 μL of double distilled water, mix well. The prepared solution should be prepared on spot. Keep chromogenic working solution protected from light on ice during use. The

prepared solution should be used up within 4 h.

④ Preparation of 10 μmol/L standard solution：

Dilute 5 μL of 2 mmol/L standard and 995 μL of buffer solution, mix well. The prepared solution should be prepared on spot. Keep 10 μmol/L standard solution protected from light on ice during use. The prepared solution should be used up within 2 h.

⑤ Preparation of reaction working solution：

Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 1255 μL of reaction working solution (mix well 1250 μL of buffer solution and 5 μL of accelerant). Keep reaction working solution protected from light on ice during use. The prepared solution should be used up within 4 h.

⑥ Preparation of COX-1 inhibitor working solution：

Before testing, please prepare sufficient COX-1 inhibitor working solution according to the test wells. For example, prepare 80 μL of COX-1 inhibitor working solution (mix well 75 μL of buffer solution and 5 μL of 1 mmol/L COX-1 Inhibitor). The prepared solution should be prepared on spot. Store at -20°C for 1 day protected from light.

⑦ Preparation of COX-2 inhibitor working solution：

Before testing, please prepare sufficient COX-2 inhibitor working solution according to the test wells. For example, prepare 80 μL of COX-2 inhibitor working solution (mix well 75 μL of buffer solution and 5 μL of 1 mmol/L COX-2 inhibitor,). The prepared solution should be prepared on spot. Store at -20°C for 1day protected from light.

⑧ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 20 μmol/L standard solution with buffer solution to a serial

concentration. The recommended dilution gradient is as follows: 0, 0.4, 0.6, 0.8, 1.2, 1.6, 1.8, 2.0 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**0.9**	**1.0**
10 μmol/L standard (μL)	0	10	15	20	30	40	45	50
Buffer solution (μL)	500	490	485	480	470	460	455	450

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL buffer solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Rat liver tissue homogenate	1-2
10% Rat kidney tissue homogenate	1-2

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: add 185 μL of standards with different concentrations to the corresponding wells.

COX well: add 15 μL of sample to the corresponding wells.

COX-1 well: add 15 μL of sample to the corresponding wells.

COX-2 well: add 15 μL of sample to the corresponding wells.

Control well: add 15 μL of buffer solution to the corresponding wells

② Add 70 μL of reaction working solution to COX well, COX-1 well, COX-2 well and control well.

③ Add 30 μL of buffer solution COX well and control well. Add 30 μL of COX-2 inhibitor working solution to COX-1 well. Add 30 μL of COX-1 inhibitor working solution to COX-2 well.

④ Mix fully with microplate reader for 3 s and incubate at 37℃ for 15 min.

⑤ Add 20 μL of chromogenic working solution to COX well, COX-1 well, COX-2 well and control well.

⑥ Add 50 μL of substrate working solution to COX well, COX-1 well, COX-2 well and control well.

⑦ Mix fully with microplate reader for 3 s and incubate at 37℃ for 10 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

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted F value.

3. Plot the standard curve by using absoluted F value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. COX activity in tissue sample:

Definition: The amount of COX in 1 g tissue protein per minute that hydrolyze the substrate to produce 1 μmol resorufin at 37℃ is defined as 1 unit.

$$\text{COX activity (U/gprot)} = (F_{sample} - F_{control} - b) \div a \div T \div C_{pr} \times 185 \div 15 \times f$$

2. COX-1 activity in tissue sample:

Definition: The amount of COX-1 in 1 g tissue protein per minute that hydrolyze the substrate to produce 1 μmol resorufin at 37℃ is defined as 1 unit.

$$\text{COX-1 activity (U/gprot)} = (F_{sample\ 1} - F_{control} - b) \div a \div T \div C_{pr} \times 185 \div 15 \times f$$

3. COX-2 activity in tissue sample:

Definition: The amount of COX-2 in 1 g tissue protein per minute that hydrolyze the substrate to produce 1 μmol resorufin at 37℃ is defined as 1 unit.

$$\text{COX-2 activity (U/gprot)} = (F_{sample\ 2} - F_{control} - b) \div a \div T \div C_{pr} \times 185 \div 15 \times f$$

[Note]

F_{sample}: The fluorescence value of COX well.

$F_{sample\ 1}$: The fluorescence value of COX-1 well.

$F_{sample\ 2}$: The absolute fluorescence value of COX-2 well.

$F_{control}$: The fluorescence value of control well.

T: The time of reaction, 10 min.

f: Dilution factor of sample before tested.

Cpr: The concentration of protein in sample, gprot/L

185: Total volume of reaction, 185 μL.

15: Sample volume, 15 μL.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	3.40	3.80	4.50
%CV	3.5	4.2	5.0

Inter-assay Precision

Three mouse liver samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	3.40	3.80	4.50
%CV	5.0	6.9	7.0

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 105%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	3.4	3.8	4.5
Observed Conc. (μmol/L)	3.46	3.8	4.72
Recovery rate (%)	102	100	105

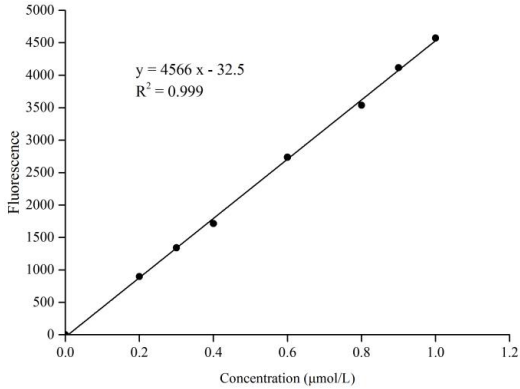
Sensitivity

The analytical sensitivity of the assay is 0.03 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the F value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	0	0.2	0.3	0.4	0.6	0.8	0.9	1.0
F	8	921	1354	1731	2755	3564	4122	4562
	6	889	1344	1711	2731	3526	4128	4602
Average F	7	905	1349	1721	2743	3545	4125	4582
Absoluted F	0	898	1342	1714	2736	3538	4118	4575



Appendix II Example Analysis

Example analysis:

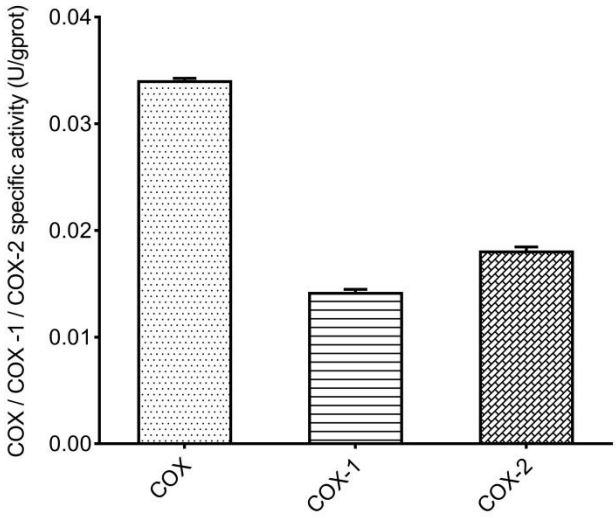
For 10% mouse liver tissue homogenate, take 15 μL of sample and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 4566 x -32.5, the average F value of COX is 1007, the average F value of COX-1 is 625, the average F value of COX-2 is 706, and the calculation result is:

$$\text{COX activity (U/gprot)} = (1007 - 364 + 32.5) \div 4566 \div 10 \div 10.65 \times 185 \div 15 \times 2 = 0.034 \text{ U/gprot}$$

$$\text{COX-1 activity (U/gprot)} = (625 - 364 + 32.5) \div 4566 \div 10 \div 10.65 \times 185 \div 15 \times 2 = 0.014 \text{ U/gprot}$$

$$\text{COX-2 activity (U/gprot)} = (706 - 364 + 32.5) \div 4566 \div 10 \div 10.65 \times 185 \div 15 \times 2 = 0.018 \text{ U/gprot}$$



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.