Amplite® Fluorometric Cyclooxygenase-2 (COX-2) Activity Assay kit *Red Fluorescence*

Catalog number: 15253
Unit size: 100 tests

Component	Storage	Amount (Cat No. 15253)
Component A: COX-2 Probe	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: COX-2 Assay Buffer	Freeze (< -15 °C)	20 ml
Component C: COX-2 Positive Control	Freeze (< -60 °C)	1 vial (25 µl)
Component D: Arachidonic Acid	Freeze (< -15 °C)	1 vial (20 µl)
Component E: Cofactor	Freeze (< -15 °C)	1 vial (20 µl)
Component F: Substrate Diluent	Freeze (< -15 °C)	1 vial (50 µl)
Component G: Resorufin Standard	Freeze (< -15 °C)	1 vial (20µl)
Component H: COX-2 Inhibitor	Freeze (< -15 °C)	1 vial (50µl)

OVERVIEW

The Amplite® Fluorometric Cyclooxygenase-2 (COX-2) Activity Assay kit provides a one-stop solution for measuring cyclooxygenase-2 (COX-2) activity and screening inhibitors of COX-2. Cyclooxygenase-2 is one of the two isoenzymes of cyclooxygenase, with COX-1 being constitutively expressed in many tissues. COX-2 is an inducible enzyme that converts arachidonic acid into prostaglandins, which are lipid mediators involved in inflammation, pain, and cancer. COX-2 is a key target in anti-inflammatory and anti-cancer drug research.

This kit uses a fluorescence detection system to measure COX-2 activity. The enzyme catalyzes the conversion of arachidonic acid into prostaglandins, generating a fluorogenic product that results in a fluorescence signal (Ex/Em = 540/590 nm). The signal generated is directly proportional to the enzymatic activity, enabling accurate quantification. This kit provides a simple and reliable solution for detecting COX-2 activity in a wide range of biological samples, including cell lysates, serum, purified enzymes, and tissue homogenates. It is compatible with fluorescence microplate readers and is ideal for studying enzyme kinetics or disease-related pathways involving COX-2 and for screening COX-2 inhibitors.

AT A GLANCE

Protocol summary

1. prepare test samples along with COX-2 positive control, resorufin standard and COX-2 inhibitor
2. Add COX-2 working solution (50 µL) in the test sample, positive control and inhibitor well.
3. Incubate at 10–30 minutes at RT.
4. Monitor fluorescence intensity at Ex/Em = 540 nm/ 590 nm (Cutoff = 570 nm).

Important notes
Store Component C (COX-2 Positive Control) at <-65 oC when received the kit.
Thaw all the kit components (except COX-2 Positive Control thaw at 4 oC) at room temperature before starting the experiment.

KEY PARAMETERS

Instrument: Fluorescence microplate reader
Excitation: 540 nm
Emission: 590 nm
Cutoff: 570 nm
Recommended plate: Solid black

PREPARATION OF STOCK SOLUTIONS

COX-2 ACTIVITY ASSAY:

COX-2 Positive Control:
Add 4 µL COX-2 Positive Control (Components C) to 100 µL of COX-2 Assay Buffer (Component B) to make COX-2 positive control solution. Mix well by pipetting up and down. Do not store diluted COX-2 solution.

Arachidonic Acid Stock Solution:
Mix 1:1 ratio of Arachidonic Acid (Component D) and Substrate Diluent (Component F) (For example: 10 µL Component D mix with 10 µL Component F). This substrate solution should be prepared fresh before the experiment.

COX-2 Probe Stock Solution:
Add 60 µL DMSO to the vial of Component A to make 100X COX-2 probe stock solution.
The COX-2 probe stock solution should be aliquoted and stored at -20°C after preparation. Avoid repeated freeze-thaw cycles.

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

COX-2 INHIBITOR ASSAY:

Prepare COX-2 Enzyme Solution:

Add 4µL COX-2 Positive Control (Components C) to 100 µL of COX-2 Assay Buffer (Component B) to make COX-2 Enzyme Solution. Mix well by pipetting up and down.do not store diluted COX-2 solution.

COX-2 Inhibitor Standard Curve:

Add 5 µL of Celecoxib (Component H) to 495 µL of COX-2 Assay Buffer (Component B) to make 100 µM of Inhibitor standard solution (Std7). Take 250 µL (Std7) and perform 1:2 serial dilutions in COX-2 Assay Buffer (Component B) to get serially diluted Inhibitor Standards (Std6-Std1).

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15253

Preparation of Resorufin Standard

Add 2 µL of Resorufin Standard (Component G) to 998 µL of COX-2 Assay Buffer (Component B) to make 10 µM of Resorufin standard solution (Std7). Take 500 µL (Std7) and perform 1:2 serial dilutions in COX-2 Assay Buffer (Component B) to get serially diluted Resorufin Standards (Std6-Std1).

PREPARATION OF WORKING SOLUTION

COX-2 Working Solution:

Add the following components to 5 mL COX-2 Assay Buffer (Component B) in this sequence:

Add 50 µL of COX-2 Probe Stock Solution (Component A), mix well, and then add 20 µL of Arachidonic Acid Stock Solution, mix well, and lastly add 10 µL Cofactor (Component E).

Note:
This COX-2 working solution should be prepared freshly before the experiment, and kept from light.
5mL COX-2 Working Solution is for 100 tests; please prepare the amount of COX-2 Working Solution as needed proportionally.

SAMPLE EXPERIMENTAL PROTOCOL

Sample COX-2 Activity Assay Protocol:

Table 1. Layout of Resorufin standards and test samples in a solid black 96-well microplate. STD = Resorufin Standards (STD1-STD7, 0.15 to 10 µM), BL= Blank Control, TS = Test Samples.

BL	BL	TS	TS
STD 1	STD 1	COX-2 Positive Control	COX-2 Positive Control
STD 2	STD 2	...	...
STD 3	STD 3		
STD 4	STD 4		
STD 5	STD 5		
STD 6	STD 6		
STD 7	STD 7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
STD1-STD7	50 µL	Serial Dilutions (0.15 to 10 µM)
BL	50 µL	COX-2 Assay Buffer (Component B)
TS	50 µL	Test Sample
COX-2 Positive Control	50 µL	COX-2 Positive Control solution

1. Prepare Resorufin standards (STD1-7), blank controls (BL), COX-2 Positive Control and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of COX-2 Working Solution to each well of Test sample, blank control, and COX-2 Positive Control. For a 384-well plate, add 25 µL of COX-2 Working Solution into each well instead.
3. Immediately after addition of the COX-2 Working Solution, measure the fluorescence (RFU) using the preset plate reader settings (Ex = 540 nm/Em = 590 nm, Cutoff=570nm) in kinetic mode reading every 30 seconds for a total of 20 minutes at room temperature. The Standard Curve can be read together with the sample or at the end of the incubation time.

Sample COX-2 Inhibitor Assay Protocol:

Table 3. Layout of Inhibitor standards in a solid black 96-well microplate. STD=Inhibitor (STD1-STD7, 0.15 to 10 µM), BL=Blank Control, TS=Test Samples.

BL	BL	...	...
STD 1	STD 1		
STD 2	STD 2		
STD 3	STD 3		
STD 4	STD 4		
STD 5	STD 5		
STD 6	STD 6		
STD 7	STD 7		

1. Prepare 40 µL COX-2 Enzyme Working solution to (STD1-7), blank controls (BL) according to the layout provided in Table 3. For a 384-well plate, add 20 µL of reagent per well instead of 40 µL.
2. Add 10 µL inhibitor solutions to each well, and incubate 5-10 min at room temperature. For a 384-well plate, add 5 µL of reagent per well.
3. Add 50 µL of COX-2 Working Solution to each well. For a 384-well plate, add 25 µL of COX-2 Working Solution into each well instead.
4. Incubate 20 minutes at room temperature, and read fluorescence intensity at (Ex = 540 nm/Em = 590 nm, Cutoff = 570nm)

EXAMPLE DATA ANALYSIS AND FIGURES

The fluorescence reading in blank wells (with assay buffer only) is used as a control, and is subtracted from the values of those wells with the Resorufin standards and test samples. The standard curve of resorufin is shown in Figure 1.

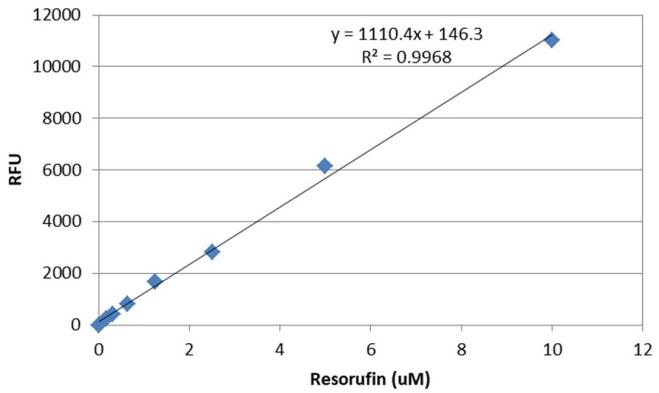

Figure 1. Dose response of typical Resorufin standard measured with Amplite™ Fluorimetric COX-2 Activity Assay Kit on a 96-well solid black microplate using a Gemini microplate reader (Molecular Devices) at Ex/Em=540 nm/590 nm (Cutoff= 570 nm).

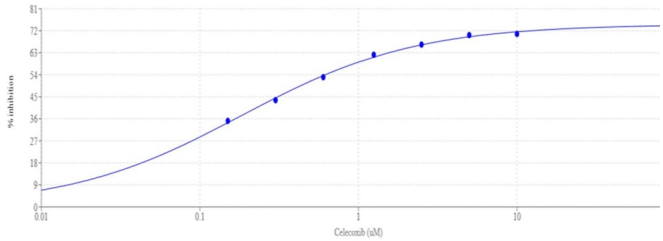

Figure2. COX-2 activity inhibition by COX-2 inhibitor.

DISCLAIMER

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