

Description

The COX1 Inhibitor Screening Assay Kit is designed to measure COX1 (cyclooxygenase 1) activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough purified recombinant COX1, Amplex™ Red, Arachidonic Diluent, COX Assay Buffer, and 100% ethanol for 100 enzyme reactions.

Background

COX1 (cyclooxygenase 1), also known as prostaglandin-endoperoxide synthase 1 (PTGS), is a peroxidase involved in the formation of prostaglandins by converting arachidonic acid to prostaglandin H₂. COX1 is expressed constitutively in most cells and participates in tissue homeostasis. COX-1 promotes cytoprotection of the gastric mucosa by increasing the formation of mucus and bicarbonate, reducing acid and pepsin secretion and keeping appropriate blood flow. It is involved in the formation of thromboxane A₂ (TxA₂) in platelets, which stimulates aggregation and vasoconstriction. COX1 and COX2 differ in position 523, with COX2 having a valine and COX1 an isoleucine residue, making the development of specific inhibitors a challenge. The use of COX inhibitors or NSAIDs (non-steroidal anti-inflammatory drugs) is common to manage pain and inflammation. Classical NSAIDs, such as aspirin and ibuprofen, can result in damage to the gastrointestinal system. The use of low dose aspirin can help prevent atherothrombosis and the discovery of other compounds able to inhibit COX1 may prove beneficial as adjuvants in cardiovascular disease therapy.

Applications

Study of enzyme kinetics and screening small molecule inhibitors for drug discovery and high-throughput screening applications.

Supplied Materials

Catalog #	Name	Amount	Storage
71110	COX1, FLAG-His-Tags*	70 µg	-80°C
	Amplex™ Red	100 µl	-80°C
	Arachidonic Diluent	250 µl	-80°C
	COX Assay Buffer	10 ml	-80°C
	100% Ethanol	1 ml	-80°C
79685	96-well black microplate	1	Room Temp

* The concentration of protein is lot-specific and will be indicated on the tube containing the protein.

Materials Required but Not Supplied

- Arachidonic Acid (Cayman Chemical #90010.1)
- Adjustable micropipettor and sterile tips
- Fluorescence plate reader capable of measurement at $\lambda_{ex}535/\lambda_{em}590$ nm

Stability

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Negative Control”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Valdecoxib as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1 x, 1 x and 10 x the IC₅₀ value shown in the validation data below.

1. Thaw **COX Assay Buffer** and **Amplex™ Red**.
2. Thaw **COX1** on ice. Briefly spin the tube to recover its full content.
3. Dilute COX1 to 35 ng/μl with COX Assay Buffer (you need 20 μl/well).
4. Add 20 μl of diluted COX1 to all wells, except “Negative Control” wells.
5. Add 70 μl of COX Assay Buffer to the “Negative Control” wells.
6. Prepare the Test inhibitor (10 μl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 100 μl.

6.1. If the Test Inhibitor is water-soluble, prepare serial dilutions in the COX Assay Buffer at concentrations 10-fold higher than the desired final concentrations.

For the positive and negative controls, use COX Assay Buffer (Diluent Solution).

OR

6.2. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in COX Assay Buffer to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

Using the COX Assay Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

For positive and negative controls, prepare 10% DMSO in COX Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO in the assay should not exceed 1%.

7. Add 10 μ l of Test inhibitor to each well designated “Test Inhibitor”.
8. Add 10 μ l of Diluent Solution to the “Positive Control” and “Negative Control” wells.
9. Dilute Amplex™ Red 10-fold with distilled water.
10. Add 10 μ l of diluted Amplex™ Red to all wells.
11. Prepare arachidonic acid (not provided) in glass vials, as follows:
 - 11.1 Prepare a 5 mM stock solution of arachidonic acid in 100% Ethanol.
 - 11.2 Perform a 1:1 dilution of 5 mM arachidonic acid with Arachidonic Diluent to obtain 2.5 mM arachidonic acid.
 - 11.3 Dilute 2.5 mM arachidonic acid 5-fold with distilled water, to prepare a 0.5 mM solution.

Component	Negative Control	Positive Control	Test Inhibitor
COX Assay Buffer	70 μ l	50 μ l	50 μ l
Test inhibitor	-	-	10 μ l
Diluent Solution	10 μ l	10 μ l	-
Diluted COX1 (35 ng/ μ l)	-	20 μ l	20 μ l
Amplex™ Red	10 μ l	10 μ l	10 μ l
Diluted Arachidonic Acid (0.5 mM)	10 μ l	10 μ l	10 μ l
Total	100 μl	100 μl	100 μl

12. Add 10 μ l of 0.5 mM arachidonic acid to all wells.
13. Immediately read the fluorescence intensity of the samples ($\lambda_{excitation}$ = 535 nm; $\lambda_{emission}$ = 590 nm) in a fluorescence reader.

Example Results

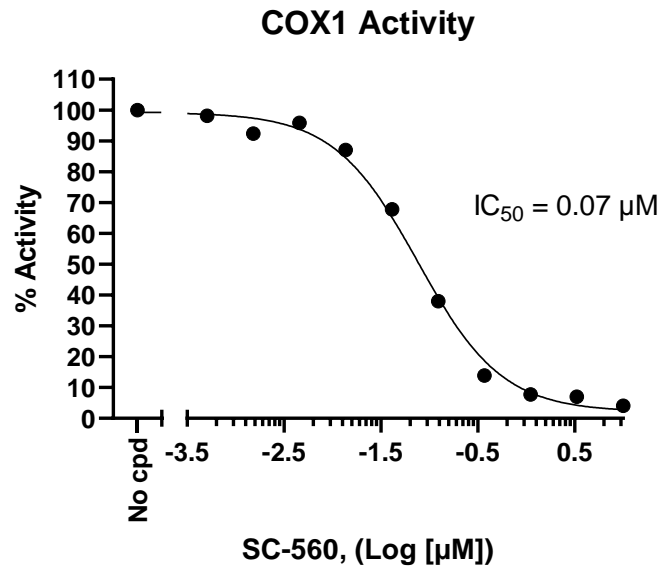


Figure 1: Inhibition of COX1 activity by SC-560.

COX1 activity was measured in the presence of increasing concentrations of SC-560 (Tocris #1550).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

Zaragoza C., et al., 2022 *Molecules* 27(3):1146.
 Bruno A., et al., 2023 *Adv Pharmacol* 97:133-165.

Related Products

Products	Catalog #	Size
COX2, FLAG-His-Tags Recombinant	71111	100 µg
COX2 Inhibitor Screening Assay Kit	82210	96 reactions
GDF15:GFRAL[Biotinylated] Inhibitor Screening Assay Kit	78807	96 reactions

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