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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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COX2 Inhibitor Screening Assay Kit

#82210
96 reactions

Description

The COX2 Inhibitor Screening Assay Kit is designed to measure COX2 (cyclooxygenase 2) activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough purified recombinant COX2, Amplex™ Red, Arachidonic Diluent, COX Assay Buffer, and 100% ethanol for 100 enzyme reactions.

Background

COX2 (cyclooxygenase 2), also known as prostaglandin-endoperoxide synthase (PTGS), is a peroxidase involved in the formation of prostaglandin during inflammation. COX2 converts arachidonic acid to prostaglandin H2. 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. While COX1 is expressed constitutively in most cells, COX2 is expressed in response to pro-inflammatory signals. Prostaglandins are involved in inflammation and pain, and the use of COX inhibitors or NSAIDs (non-steroidal anti-inflammatory drugs) is common to manage these symptoms. Classical NSAIDs, such as aspirin and ibuprofen, are not COX2 specific and can result in damage to the gastrointestinal system. Recently the focus has been on developing selective inhibitors, such as celecoxib and etoricoxib, which can result in less stomach ulceration. These, however, can cause heart failure and other cardiovascular abnormalities. In the central nervous system COX2 is found in normal conditions and contributes to synaptic activity and other brain related activities, and COX2 inhibition may prevent brain inflammation and neurodegeneration. COX2 is also upregulated in cancers, such as breast, lung, colon, cervical and pancreatic cancer. Prostaglandin H2 can be converted into PGE2 (prostaglandin E2), that can result in increased VEGF (vascular endothelial growth factor) levels, expression of BCL2 (B cell lymphoma 2), and activation of other signaling pathways involved in cancer progression. An understanding of the role of COX2 and mode of action of its inhibitors can result in optimized therapies for COX2 related pathologies.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
71111	COX2, FLAG-His-Tags*	35 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 λex535/λem590 nm

BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

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 IC_{50} value shown in the validation data below.

1. Thaw **COX Assay Buffer** and **Amplex™ Red**.

2. Thaw **COX2** on ice. Briefly spin the tube to recover its full content.

3. Dilute COX2 to 17.5 ng/µl with COX Assay Buffer (you need 20 µl/well).

4. Add 20 µl of diluted COX2 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 µl of Test inhibitor to each well designated "Test Inhibitor".

8. Add 10 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.

9. Dilute Amplex™ Red 10-fold with distilled water (10 µl/well).

10. Add 10 µl 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. This makes a 0.5 mM solution (10 µl/well).

Component	**Negative control**	**Positive Control**	**Test Inhibitor**
COX Assay Buffer	70 µl	50 µl	50 µl
Test inhibitor	-	-	10 µl
Diluent Solution	10 µl	10 µl	-
Diluted COX2 (17.5 ng/µl)	-	20 µl	20 µl
Amplex™ Red	10 µl	10 µl	10 µl
Diluted Arachidonic Acid (0.5 mM)	10 µl	10 µl	10 µl
Total	**100 µl**	**100 µl**	**100 µl**

12. Add 10 µl of 0.5 mM arachidonic acid to all wells.

13. Immediately read the fluorescence intensity of the samples (lexcitation = 535 nm; lemission = 590 nm) in a fluorescence plate reader.

Example Results

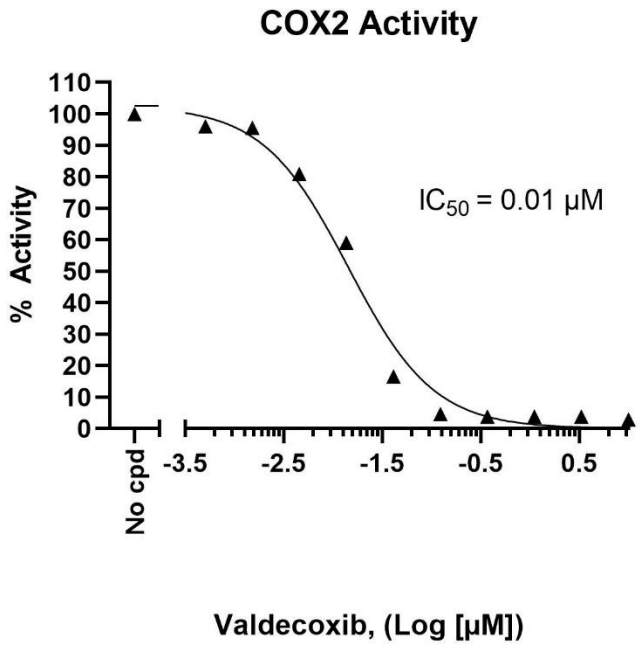

Figure 1: Inhibition of COX2 activity by Valdecoxib.
COX2 activity was measured in the presence of increasing concentrations of Valdecoxib (SelleckChem #S4049).

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
Minghetti L., 2004 *J Neuropathol Exp Neurol* 63(9): 901-10.
Giovannini M., *et al.*, 2003 *Int J Immunopathol Pharmacol* 16(2):31-40.
Pang L., *et al.*, 2016 *Stem Cells Int* 2016:2048731.

Related Products

Products	*Catalog #*	*Size*
COX1, FLAG-His-Tags Recombinant	71110	100 µg/1 mg
BCL-2 TR-FRET Assay Kit	50222	96 reactions/384 reactions
BCL-2, His-tag Recombinant	50272	100 µg
Human VEFG165 (E. coli derived) Recombinant	79516	10 µg

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