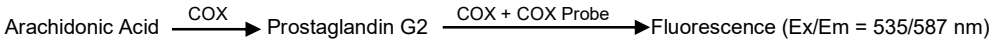
COX-1 Inhibitor Screening Kit (Fluorometric) (BN00778)

(Catalog # BN00778; 100 assays; Store at -20°C)

I. Introduction:

Cyclooxygenase (COX), also known as prostaglandin-endoperoxide synthase (PTGS, EC 1.14.99.1), is an enzyme that is responsible for the formation of important biological mediators called prostanoids, including prostaglandins, prostacyclin and thromboxane. COX is the central enzyme in the biosynthetic pathway to prostanoids from arachidonic acid. There are two known isoenzymes: COX-1 and COX-2. COX-1 is constitutively expressed in many tissues and is the predominant form in gastric mucosa and in kidney. COX-2 is not expressed under normal conditions in most cells, but elevated levels are found during inflammation. Pharmacological inhibition of COX by non-steroidal anti-inflammatory drugs (NSAID) can provide relief from the symptoms of inflammation and pain. Assay Genie's COX-1 Inhibitor Screening Kit offers a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of COX-1 inhibitors. The assay is based on the fluorometric detection of Prostaglandin G2, the intermediate product generated by the COX enzyme.



II. Application:

- Screening/studying/characterizing COX-1 inhibitors.

III. Kit Contents:

Components	BN00778	Cap Code	Part Number
COX Assay Buffer	25 ml	WM	BN00778-1
COX Probe (in DMSO)	200 µl	Red	BN00778-2A
COX Cofactor (in DMSO)	20 µl	Violet	BN00778-3
Arachidonic Acid	50 µl	Blue	BN00778-4
NaOH	500 µl	Clear	BN00778-5
COX-1, Ovine	1 vial	Green	BN00778-6
SC560, COX-1 inhibitor (in DMSO)	100 µl	Orange	BN00778-7

IV. User Supplied Reagents and Equipment:

- 96-well white opaque plate with flat bottom.
- Multi-well spectrophotometer (fluorescence plate reader)
- Multi-channel pipette (adjustable to 10 µl)
- DMSO

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay. Unless specified, bring assay components to room temperature (RT) before use.

- **COX-1:** Reconstitute with 110 µl of sterile ddH_2O. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Use within two months. For short-term storage (~ 2 weeks), COX-1 can be stored at -20°C. Keep on ice while in use. It's stable for at least ~30 min. on ice. **Note:** we recommend not keeping the enzyme on ice for long.

VI. COX-1 inhibitor Screening Protocol:

1. **Screening Compounds, Inhibitor Control, and Enzyme Control Preparations:** Dissolve test inhibitors in proper solvent (e.g. DMSO). Dilute to 10X the desired test concentration with COX Assay Buffer before use. Add 10 µl diluted test inhibitor or Assay Buffer into assigned wells as sample screen [S] or Enzyme Control [EC] (no inhibitor) respectively. Add 2 µl of SC560 and 8 µl COX Assay Buffer into one of the wells as Inhibitor Control [IC].

   Note: Solvents used to solubilize the inhibitors might affect the enzymatic activity. If solvent effect on enzymatic activity is a concern, prepare a solvent control well with the same final concentration of the solvent as in the inhibitor sample as solvent control.

2. **Reaction Preparation:** Dilute COX Cofactor 200 times by adding 2 µl of COX Cofactor to 398 µl of COX Assay Buffer just before use. Mix well. Prepare Arachidonic Acid solution by adding 5 µl of supplied Arachidonic Acid to 5 µl of NaOH just before use. Vortex briefly to mix. Dilute Arachidonic Acid/NaOH solution 10 times by adding 90 µl ddH_2O, vortex briefly to mix. Make as much as needed.
   For each well, prepare 80 µl of master mix as follows:

	Reaction Master Mix
COX Assay Buffer	76 µl
COX Probe	1 µl
Diluted COX Cofactor	2 µl
COX-1	1 µl

   Add 80 µl of Reaction Mix into each well. Use a multi-channel pipette to add 10 µl of diluted Arachidonic Acid/NaOH solution into each well to initiate all the reactions at the same time.

   Notes:

   a. Diluted COX Cofactor is stable for 1 hr at RT. We don't recommend storing the diluted COX Cofactor.

   b. Diluted Arachidonic Acid/NaOH solution is stable for at least 1 hr on ice. We don't recommend storing diluted Arachidonic Acid/NaOH solution.

c. Preset the plate reader to avoid delay in measurement after addition of Arachidonic Acid/NaOH solution.

3. **Measurement:** Measure fluorescence (Ex/Em = 535/587 nm) kinetically at 25°C for 5-10 min. Choose two points (T_1 and T_2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU_1 and RFU_2).
4. **Calculation:** Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU ($RFU_2 - RFU_1$) values by the time ΔT ($T_2 - T_1$). Calculate % Relative Inhibition as follows:

$$\text{\% Relative Inhibition} = \frac{\text{Slope of EC} - \text{Slope of S}}{\text{Slope of EC}} \text{X100}$$

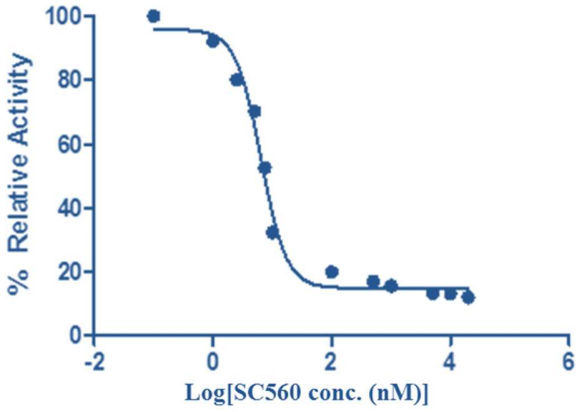


Figure: Inhibition of COX-1 Activity with SC560. IC_{50} of SC560 was determined to be 6.45 nM. Assay was performed following the kit protocol.

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