Technical Bulletin

COX-2 Inhibitor Screening Kit (Fluorometric)

Catalog Number MAK399

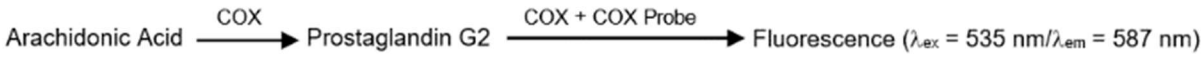
Product Description

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

The COX-2 Inhibitor Screening Kit offers a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of COX-2 inhibitors. The assay is based on the fluorometric detection of Prostaglandin G_2, the intermediate product generated by the COX enzyme. The COX Probe produces a fluorescent signal (λ_{Ex} = 535 nm/ λ_{Em} = 587 nm) proportional to the Prostaglandin G_2 generated by the COX-2 enzyme.

The kit is suitable for screening, studying and characterizing COX-2 inhibitors.



Components

The kit is sufficient for 100 fluorometric assays in 96 well plates.

- COX Assay Buffer 25 mL
  Catalog Number MAK399A
- COX Probe (in DMSO) 200 µL
  Catalog Number MAK399B
- COX Cofactor (in DMSO) 20 µL
  Catalog Number MAK399C
- Arachidonic Acid 1 vial
  Catalog Number MAK399D
- NaOH 500 µL
  Catalog Number MAK399E
- COX-2, Human Recombinant 1 vial
  Catalog Number MAK399F
- Celecoxib, 100 µL
  COX-2 inhibitor (in DMSO)
  Catalog Number MAK399G

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multi-channel pipettor)
- Fluorescence multiwell plate reader
- White or opaque flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Anhydrous Dimethyl sulfoxide (DMSO) (Catalog Number 276855 or equivalent)
- Ethanol, 200 Proof (Catalog Number E7023 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Unless specified, bring assay components to room temperature prior to use.

COX-2, Human Recombinant: Reconstitute the vial with 110 µL of purified water. Aliquot and store at -80 °C. Avoid repeated freeze/thaw cycles. Use within two months of reconstitution. For short-term storage (~ 2 weeks), COX-2 can be stored at -20 °C. Keep on ice while in use; Solution is stable for ~30 minutes on ice. It is **not** recommended to keep the enzyme on ice for periods longer than 30 minutes.

Arachidonic Acid: Reconstitute the vial in 55 µL of 100% Ethanol (not included) and vortex for 15-30 seconds.

Procedure

Screening Compound Preparation (S)

1. Dissolve test inhibitors in proper solvent (e.g. DMSO, not included).
2. Dilute solution from Step 1 to 10× of the desired test concentration with COX Assay Buffer before use.
3. Add 10 µL of diluted test inhibitor from Step 2 into assigned well(s) as Sample screen (S).

Enzyme Control (no inhibitor) (EC)

Add 10 µL of Assay Buffer into assigned well as Enzyme Control (EC).

Inhibitor Control (IC)

Add 2 µL of Celecoxib, COX-2 inhibitor and 8 µL of COX Assay Buffer into assigned well as Inhibitor Control (IC).

Solvent Control (SC)

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

Reaction Preparation

1. Preset the plate reader to 25 °C, kinetic mode, λ_{Ex} = 535 nm/λ_{Em} = 587 nm to avoid delay in measurement after addition of Arachidonic Acid/NaOH solution.
2. Immediately prior to use, dilute COX Cofactor 200× by adding 2 µL of COX Cofactor to 398 µL of COX Assay Buffer, mix well. Diluted COX Cofactor is stable for 1 hour at room temperature. **Do not store diluted solution**.

3. Immediately prior to use, prepare Arachidonic Acid solution by adding 5 µL of reconstituted Arachidonic Acid to 5 µL of NaOH. Vortex briefly to mix. Further dilute Arachidonic Acid/NaOH solution 10× by adding 90 µL of purified water to the same vial, and vortex briefly to mix. Prepare as much as needed. Diluted Arachidonic Acid/NaOH is stable for 1 hour at room temperature. **Do not store diluted solution**.
4. Mix enough reagents for the number of assays to be performed. For each well, prepare 80 µL of Reaction Mix according to Table 1.

Table 1.
Reaction Mix Preparation

Reagent	Volume
COX Assay Buffer	76 µL
COX Probe	1 µL
Diluted COX Cofactor	2 µL
COX-2, Human Recombinant	1 µL

5. Add 80 µL of Reaction Mix into each well.
6. Using a multi-channel pipette, add 10 µL of diluted Arachidonic Acid/NaOH solution into each well to initiate the reactions at the same time.

Measurement

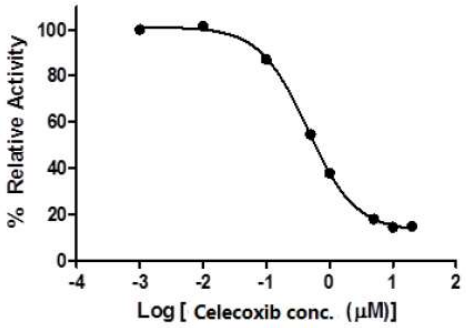
Immediately measure the fluorescence at λ_{Ex} = 535 nm/λ_{Em} = 587 nm kinetically at 25 °C for 5-10 minutes.

Results

1. 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).
2. Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU ($RFU_2 - RFU_1$) values by the time ΔT ($T_2 - T_1$).
3. If the fluorescent signal from the Solvent Control is significant, subtract the slope of the Solvent Control (SC) from the slope of the Sample (S).
4. Calculate % Relative Inhibition as follows:

$$\frac{\text{Slope of EC} - \text{Slope of S}}{\text{Slope of EC}} \times 100\%$$

Figure 1.
Inhibition of COX-2 Activity with Celecoxib, COX-2 inhibitor. IC50 of Celecoxib was determined to be 0.45 µM. Assay was performed following the kit protocol.



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