ab283401– COX2 Inhibitor Screening Kit (Fluorometric)

For the screening of potential COX2 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283401

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. COX2 Inhibitor Screening Kit (Fluorometric) (ab283401) offers a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of COX-2 inhibitors. This assay is based on the fluorometric detection of Prostaglandin G2, the intermediate product generated by the COX enzyme.

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
COX Assay Buffer	25 mL	-20°C
OxiRed™ Probe	0.2 mL	-20°C
COX Cofactor	20 µl	-20°C
Arachidonic Acid	1 vial	-20°C
NaOH Solution	500 µl	-20°C
Human Recombinant COX-2	1 vial	-20°C
Celecoxib (COX-2 Inhibitor)	100 µl	-20°C

PLEASE NOTE: OxiRed™ Probe was previously labelled as OxiRed Probe and COX Probe (in DMSO). The composition has not changed.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Multi-well spectrophotometer (fluorescence plate reader)
- 96-well white opaque plate with flat bottom
- Multi-channel pipette (adjustable to 10 µl)
- DMSO
- 100% Ethanol

Δ Note: The use of 96-well white plates could lead to fluorescence signal saturation in certain instruments. Reduce the fluorescence gain setting or use a 96-well black plate with flat bottom.

Reagent Preparation

- Before using the kit, spin the tubes prior to opening. Unless specified, bring assay components to room temperature (RT) before use

Human Recombinant COX-2: Reconstitute the vial with 110 µl of sterile ddH_2O. Aliquot and store at -80°C. Avoid repeated freeze/thaw cycles. Use within two months. For short-term storage (about 2 weeks), COX2 can be stored at -20°C. Keep on ice while in use. It's stable for at least ~30 minutes on ice.

Δ Note: *Do not keep the enzyme on ice for longer periods.*

Arachidonic Acid: Reconstitute the vial in 55 µl of 100% Ethanol and vortex for 15-30 seconds.

Assay Protocol

Sample Compounds, Inhibitor Control, and Enzyme Control Preparation:

1. Dissolve test inhibitors in proper solvent (i.e. DMSO).
2. Dilute to 10X desired test concentration with COX Assay Buffer.
3. Add 10 µl diluted test inhibitor or COX Assay Buffer into assigned wells as sample screen [S] or Enzyme Control [EC] (no inhibitor) respectively.
4. Add 2 µl of Celecoxib (COX-2 Inhibitor) and 8 µl COX Assay Buffer into one of the wells as Inhibitor Control [IC].

Δ Note: *High solvent concentration might affect the enzymatic activity. Prepare parallel well(s) as Solvent Control to test the effect of the solvent on enzyme activity (same as EC in presence of final solvent concentration).*

Reaction Preparation:

1. Dilute COX Cofactor 200-fold by adding 2 µl of COX Cofactor to 398 µl of COX Assay Buffer just before use.
2. Prepare Arachidonic Acid solution by adding 5 µl of supplied Arachidonic Acid to 5 µl of NaOH Solution just before use, vortex briefly to mix.
3. Dilute Arachidonic Acid/NaOH solution 10 times by adding 90 µl ddH2O, vortex briefly to mix. Prepare enough for the number of assays to be performed. For each well, prepare 80 µl of master mix as follows:

Reaction Master Mix	Volume
COX Assay Buffer	76 µl
OxiRed Probe	1 µl
Diluted COX Cofactor	2 µl
Human Recombinant COX-2	1 µl

4. 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 the reactions at the same time.

Δ Notes:

a. *Diluted COX Cofactor is stable for 1 hour at RT. Storing diluted COX Cofactor is not recommended.*
b. *Diluted Arachidonic Acid/NaOH solution is stable for at least 1 hour on ice. Storing diluted Arachidonic Acid/NaOH solution is not recommended.*
c. *Pre-set the plate reader to avoid delay in measurement after addition of Arachidonic Acid/NaOH solution.*

Measurement

1. Measure RFU at Ex/Em = 535/587 nm in kinetic mode for 5-10 minutes at 25°C.
2. Choose any two time points (t1 and t2) in the linear range of the plot and obtain the corresponding values for the RFU (RFU1 and RFU2).

Calculations:

1. Calculate the slope for all samples, including Enzyme Control [EC].

2. Slope can be calculated by dividing the net ΔRFU (=RFU2-RFU1) value by the time Δt (=t2-t1).
3. Calculate % relative inhibition using the following equation.

$$\% \text{ Relative Inhibition} = \frac{slope\ of\ EC - Slope\ of\ S}{slope\ of\ \text{EC}} \times 100$$

Where Slope of EC is the slope of Enzyme Control and Slope S is the slope of Sample Screen

Technical Support

For all technical or commercial enquiries please go to:
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