A convenient, easy-to-follow shortened protocol is now being provided with this assay.
For a detailed protocol go to www.caymanchem.com/pdfs/700100.pdf

COX Fluorescent Inhibitor Screening Assay Kit Short Protocol

Item No. 700100

REAGENT PREPARATION

1. **Assay Buffer (10X) (Item No. 760114)** - Dilute 3 mls with 27 ml HPLC-grade water (final formulation is 100 mM Tris-HCl, pH 8.0); stable 6 months at 4°C.
2. **Heme (Item No. 760116)** - Dilute 40 µl with 960 µl diluted assay buffer; stable 12 hours at 22°C.
3. **COX-1 (ovine) (Item No. 700103)** - For approximately 48 wells, dilute 40 µl of enzyme with 440 µl of diluted Assay Buffer and store on ice; use within 1 hour.
4. **COX-2 (human recombinant) (Item No. 700104)** - For approximately 48 wells, dilute 40 µl of enzyme with 440 µl of diluted Assay Buffer and store on ice; use within 1 hour.
5. **Arachidonic Acid (Item No. 760113)** - Transfer 100 µl of the supplied solution to another vial, add 100 µl of KOH (Item No. 760115), vortex, and dilute with 800 µl of HPLC-grade water (concentration = 2 mM); use within 30 minutes.
6. **Potassium Hydroxide (Item No. 760115)** - Ready to use as supplied.
7. **DMSO (Item No. 700001)** - Ready to use as supplied.
8. **ADHP (Item No. 700002)** - Dissolve the contents of one vial with 100 µl DMSO (Item No. 700001) and then add 900 µl of diluted Assay Buffer; use within 30 minutes.
9. **DuP-697 (Item No. 760158)**; optional - Ready to use as supplied. Assaying 10 µl will yield approximately 90% inhibition or greater.
10. **SC-560 (Item No. 760159)**; optional - Ready to use as supplied. Assaying 10 µl will yield approximately 90% inhibition or greater.

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PERFORMING THE ASSAY

Pipette the reagents as indicated in the table below and incubate the reaction as described.

Wells	Assay Buffer	Heme	Enzyme	Compound Solvent	Inhibitor/ Sample
100% Initial Activity (IA) 3 wells	150 µl	10 µl	10 µl	10 µl	-
Background (IA) 3 wells	160 µl	10 µl	-	10 µl	-
Inhibitor/Sample triplicate recommended	150 µl	10 µl	10 µl	-	10 µl
Incubate inhibitor + enzyme for 5 minutes at room temperature					
Add 10 µl ADHP to each of the wells					
Quickly add 10 µl Arachidonic Acid to all wells to initiate reactions					
Incubate precisely 2 minutes at room temperature					
Read the plate at Excitation = 530-540; Emission = 585-595					

CALCULATIONS

$$\textbf{\% Inhibition} = \frac{[\text{Initial Activity} - \text{Sample Activity}]}{\text{Initial Activity}} \times 100$$

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