ab204698 – Cyclooxygenase 1 (COX1) Inhibitor Screening Assay Kit (Fluorometric)

For the screening of potential COX1 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab204698

Storage and Stability

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.

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
COX-1 Enzyme	1 vial	-20°C
SC560 (COX-1 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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

COX-1 Enzyme: Reconstitute with 110 µl of sterile ddH2O. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Use within two months. For short-term storage (~ 2 weeks), COX-1 Enzyme can be stored at -20°C. Keep on ice while in use. It's stable for at least ~30 min. on ice.
Δ Note: We recommend not keeping the enzyme on ice for long.
Arachidonic Acid: Reconstitute the vial in 55 µl of 100% Ethanol and vortex for 15-30 sec.

Screening Protocol

Screening Compounds, Inhibitor Control, and Enzyme Control Preparation:

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

Δ 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.

Version 4a, Last updated 6 June 2025

Reaction Preparation:

1. Dilute COX Cofactor 200 times by adding 2 µl of COX Cofactor to 398 µl of COX Assay Buffer just before use. Mix well.
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. Make as much as needed. For each well, prepare 80 µl of master mix as follows:

Item	Reaction Master Mix
COX Assay Buffer	76 µl
OxiRed™ Probe	1 µl
Diluted COX Cofactor	2 µl
COX-1 Enzyme	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 all the reactions at the same time.

Δ Notes:
a. Diluted COX Cofactor is stable for 1 hr at RT. We do not 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. Pre-set the plate reader to avoid delay in measurement after addition of Arachidonic Acid/NaOH solution.

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).

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:

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

Technical Support

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