Human/Mouse COX-2 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF4198

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse COX-2 in Western blots. In Western blots, less than 1% cross-reactivity with recombinant human COX-1 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human COX-2 Ala18-Leu604 Accession # P35354
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μm filtered solution in PBS.

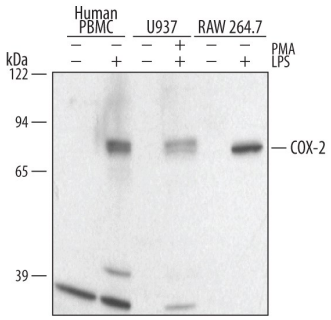
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below

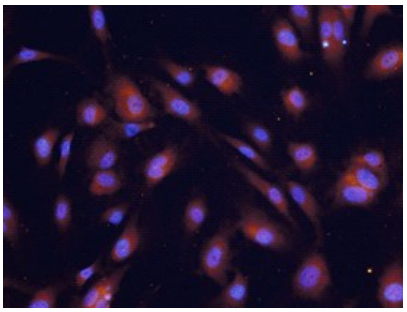
DATA

Western Blot



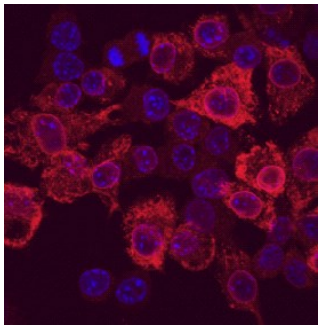
Detection of Human and Mouse COX-2 by Western Blot. Western blot shows lysates of human peripheral blood mononuclear cell (PBMC) and RAW 264.7 mouse monocyte/macrophage cell line untreated (-) or treated (+) with 1 ug/mL LPS for 24 hours and U937 human histiocytic lymphoma cell line untreated or treated with 100 nM PMA and 1 ug/mL LPS for 48 hours and 24 hours, respectively. PVDF membrane was probed with 1 µg/mL of Human/Mouse COX-2 Polyclonal Antibody (Catalog # AF4198), followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). A specific band was detected for COX-2 at approximately 75 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 2.

Immunocytochemistry



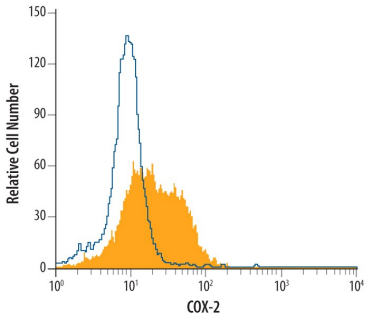
COX-2 in HUVEC Human Cells. COX-2 was detected in immersion fixed HUVEC human umbilical vein endothelial cells using 10 µg/mL Human/Mouse COX-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4198) for 3 hours at room temperature. Cells were stained with the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunocytochemistry



COX-2 in RAW 264.7 Mouse Cells. COX-2 was detected in immersion fixed RAW 264.7 mouse monocyte/macrophage cells stimulated with LPS using Goat Anti-Human/Mouse COX-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4198) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Intracellular Staining by Flow Cytometry



Detection of COX-2 in RAW 264.7 Mouse Cell Line by Flow Cytometry. RAW 264.7 mouse monocyte/macrophage cell line treated with 1 µg/mL LPS for 24 hours was stained with Goat Anti-Human/Mouse COX-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4198, filled histogram) or control antibody (Catalog # AB-108-C, open histogram), followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

Cyclooxygenase-2 (COX-2) also known as prostaglandin G/H synthase 2 (PGHS2) is a 70 kDa microsomal enzyme that belongs to the prostaglandin G/H synthase family. It is inducibly-expressed by a number of cell types, including fibroblasts, vascular smooth muscle cells, endothelium, and monocytes. Functionally, COX-2 is a homodimer that catalyzes two steps in the conversion of arachadonic acid to prostaglandin H_2. Mature human COX-2 is 587 amino acids (aa) in length and contains one EGF-like domain (aa 18-55), a potential membrane interacting region (aa 277-292) and a globular catalytic domain (aa 293-604). At least one splice form exists that shows an 11 aa substitution for the C-terminal 451 amino acids. Mature human COX-2 shows 87% aa identity to mouse COX-2.