Anti-COX2/Cyclooxygenase 2 Antibody [SC56-06]

ET1610-23

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 69 kDa
Clone number:	SC56-06

Description: Prostaglandins are a diverse group of autocrine and paracrine hormones that mediate many cellular and physiologic processes. Prostaglandin H2 (PGH2) is an intermediate molecule in formation of the prostaglandins. Cyclooxygenase-1 (Cox-1) and cyclooxygenase-2 (Cox-2) are prostaglandin synthases that catalyze the formation of PGH2 from arachidonic acid (AA). Cox-1 and Cox-2 are isozymes of prostaglandin-endoperoxidase synthase (PTGS). Cox-1 is constitutively expressed in most tissues and is thought to serve in general “housekeeping” functions. Cox-2 is efficiently induced in migratory cells responding to pro-inflammatory stimuli and is considered to be an important mediator of inflammation. Both enzymes are targets for the nonsteroidal therapeutic anti-inflammatory drugs, NSAIDs.

Immunogen: Synthetic peptide within human COX2 aa 100-140.

Positive control: NIH/3T3 cell lysate, C2C12 cell lysate, L6 cell lysate, Mouse bladder tissue lysate, Mouse small intestine tissue lysate, C2C12, A549 cell lysates, A549, rat bladder tissue, human colon tissue, mouse kidney tissue, mouse uterus tissue.

Subcellular location: Microsome membrane, Endoplasmic reticulum membrane, Nucleus inner membrane, Nucleus outer membrane.

Database links: SwissProt: P35354 Human | Q05769 Mouse | P35355 Rat

Recommended Dilutions:

WB	1:500-1:1,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:100-1:5,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

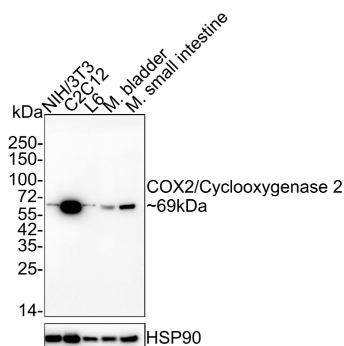

Fig1: Western blot analysis of COX2/Cyclooxygenase 2 on different lysates with Rabbit anti-COX2/Cyclooxygenase 2 antibody (ET1610-23) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate (20 µg/Lane)
Lane 2: C2C12 cell lysate (20 µg/Lane)
Lane 3: L6 cell lysate (20 µg/Lane)
Lane 4: Mouse bladder tissue lysate (40 µg/Lane)
Lane 5: Mouse small intestine tissue lysate (40 µg/Lane)

Predicted band size: 69 kDa
Observed band size: 69 kDa

Exposure time: 3 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1610-23) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

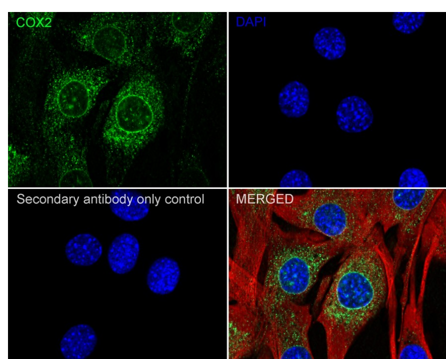

Fig2: Immunocytochemistry analysis of C2C12 cells labeling COX2/Cyclooxygenase 2 with Rabbit anti-COX2/Cyclooxygenase 2 antibody (ET1610-23) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-COX2/Cyclooxygenase 2 antibody (ET1610-23) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

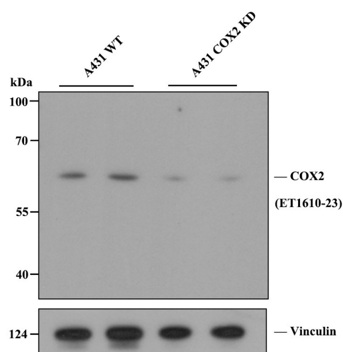

Fig3: All lanes: Western blot analysis of COX2 with anti-COX2 antibody [SC56-06] (ET1610-23) at 1:1,000 dilution.
Lane 1/2: Wild-type A431 whole cell lysate (20 µg).
Lane 3/4: COX2 fragment knockdown A431 whole cell lysate (20 µg).

ET1610-23 was shown to specifically react with COX2 in wild-type A431 cells. Weakened bands were observed when COX2 knockdown samples were tested. Wild-type and COX2 knockdown samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1610-23, 1/1,000) and Loading control antibody (Rabbit anti-Vinculin, ET1705-94, 1/5,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG-HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

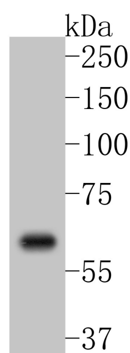

Fig4: Western blot analysis of COX2/Cyclooxygenase 2 on A549 cell lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1610-23, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

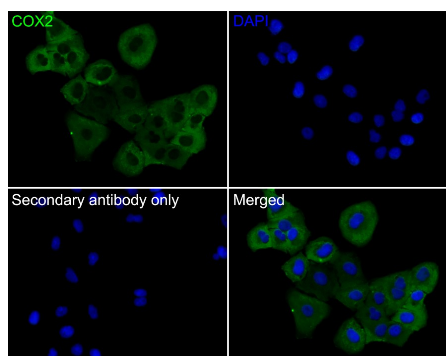

Fig5: Immunocytochemistry analysis of A549 cells labeling COX2/Cyclooxygenase 2 with Rabbit anti-COX2/Cyclooxygenase 2 antibody (ET1610-23) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-COX2/Cyclooxygenase 2 antibody (ET1610-23) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

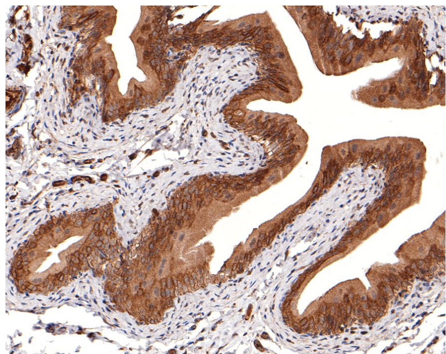

Fig6: Immunohistochemical analysis of paraffin-embedded rat bladder tissue with Rabbit anti-COX2/Cyclooxygenase 2 antibody (ET1610-23) at 1/100 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-23) at 1/100 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

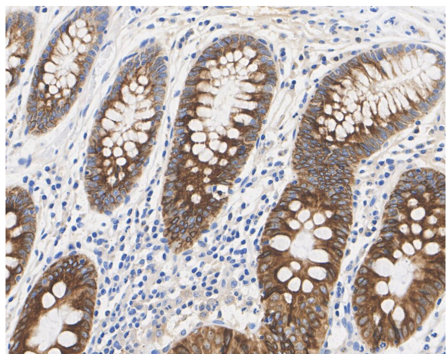

Fig7: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-COX2/Cyclooxygenase 2 antibody (ET1610-23) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-23) at 1/5,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

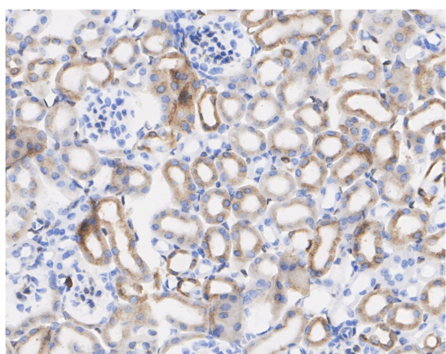

Fig8: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-COX2/Cyclooxygenase 2 antibody (ET1610-23) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-23) at 1/5,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

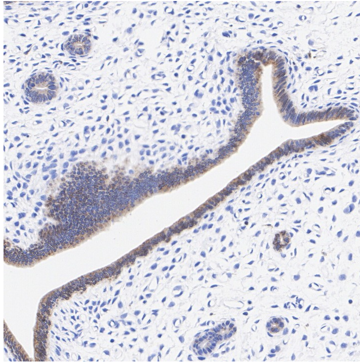

Fig9: Immunohistochemical analysis of paraffin-embedded mouse uterus tissue with Rabbit anti-COX2/Cyclooxygenase 2 antibody (ET1610-23) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-23) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Note: All products are “FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE”.

Background References

1. Kaczocha M et al. Fatty acid binding protein deletion suppresses inflammatory pain through endocannabinoid/N-acylethanolamine-dependent mechanisms. Mol Pain 11:52 (2015).
2. Wang J et al. Mechanism of QSYQ on anti-apoptosis mediated by different subtypes of cyclooxygenase in AMI induced heart failure rats. BMC Complement Altern Med 15:352 (2015).