Product datasheet

Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] ab179800

Overview

Product name   Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012]
Description    Rabbit monoclonal [EPR12012] to COX2 / Cyclooxygenase 2
Host species   Rabbit
Tested applications  Suitable for: WB, IP, ICC/IF, IHC-P
                    Unsuitable for: Flow Cyt
Species reactivity Reacts with: Mouse, Human
Immunogen       Synthetic peptide within Human COX2/ Cyclooxygenase 2 aa 550 to the C-terminus (Cysteine residue). The exact sequence is proprietary.
                    Database link: P35354
Positive control WB: A549, U-87 MG and HeLa cell lysates; mouse spleen tissue lysate. IHC-P: Human colonic carcinoma, lung carcinoma, liver and colon tissues and rat kidney tissue lysate; mouse kidney and liver tissue. IP: A549 cell lysate ICC/IF: U-87 MG cells

General notes   Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

This product is a recombinant monoclonal antibody, which offers several advantages including:
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.
Properties

Form
Liquid

Storage instructions

Storage buffer
pH: 7.20
Preservative: 0.01% Sodium azide
 Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity
Protein A purified

Clonality
Monoclonal

Clone number
EPR12012

Isotype
IgG

Applications

Our Abpromise guarantee covers the use of ab179800 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<td>1/10 - 1/100.</td>
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<td>ICC/IF</td>
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<td>Use at an assay dependent concentration.</td>
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<td>IHC-P</td>
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<td>1/100 - 1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols.</td>
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Application notes
Is unsuitable for Flow Cyt.

Target

Function
Mediates the formation of prostaglandins from arachidonate. May have a role as a major mediator of inflammation and/or a role for prostanoid signaling in activity-dependent plasticity.

Pathway
Lipid metabolism; prostaglandin biosynthesis.

Sequence similarities
Belongs to the prostaglandin G/H synthase family. Contains 1 EGF-like domain.

Post-translational modifications
S-nitrosylation by NOS2 (iNOS) activates enzyme activity. S-nitrosylation may take place on different Cys residues in addition to Cys-561.

Cellular localization
Microsome membrane. Endoplasmic reticulum membrane.

Images
**All lanes**: Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800) at 1/1000 dilution

**Lane 1**: A549 cell lysate
**Lane 2**: U-87 MG cell lysate
**Lane 3**: Wild-type HeLa cell lysate
**Lane 4**: PTGS2 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size**: 69 kDa

**Lanes 1 - 4**: Merged signal (red and green). Green - ab179800 observed at 75 kDa. Red - loading control, ab8245 observed at 37 kDa.

ab179800 was shown to react with COX2 / Cyclooxygenase 2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255420 (knockout cell lysate ab263795) was used. Wild-type and COX2 / Cyclooxygenase 2 knockout samples were subjected to SDS-PAGE. ab179800 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue sections labeling COX2 / Cyclooxygenase 2 with purified ab179800 at 1/4000 dilution (0.125 µg/ml).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: secondary antibody only control. Hematoxylin was used as a counterstain.

Immunocytochemistry/ Immunofluorescence analysis of U-87 MG (human glioblastoma-astrocytoma epithelial cell) cells labeling COX2 / Cyclooxygenase 2 with ab179800 at 1/50 dilution. ab150077 (AlexaFluor® 488 Goat anti-Rabbit) at 1/1000 was used as secondary antibody. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% TritonX-100. ab195889, Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 was used as counterstain. Nuclie were stained blue with DAPI.

Confocal image showing cytoplasmic staining in U-87 MG cell line. Negative control: MCF7 PMID: 18199541.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue sections labeling COX2 / Cyclooxygenase 2 with purified ab179800 at 1/4000 dilution (0.125 µg/ml).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: secondary antibody only control. Hematoxylin was used as a counterstain.

**Western blot**

**All lanes:** Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800) at 1/1000 dilution (Purified)

**Lane 1:** U-87 MG (human glioblastoma-astrocytoma epithelial cell line) whole cell lysate with 5% NFDM/TBST
**Lane 2:** HCT 116 (human colorectal carcinoma cell line) whole cell lysate with 5% NFDM/TBST
**Lane 3:** MCF7 (human breast adenocarcinoma cell line) whole cell lysate with 5% NFDM/TBST

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes:** Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

**Predicted band size:** 69 kDa
**Observed band size:** 72 kDa

*why is the actual band size different from the predicted?*

**Exposure time**

**Lane 1:** 3.25 seconds
**Lane 2 and 3:** 180 seconds

The expression profile observed in HCT 116 and MCF7 are consistent with the literatures (PMID: 14739610, PMID: 24325753, 5...
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colonic carcinoma tissue labelling COX2 / Cyclooxygenase 2 with unpurified ab179800 at a dilution of 1/250.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded) analysis of human liver tissue labelling COX2 / Cyclooxygenase 2 with unpurified ab179800 at a dilution of 1/250.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800) at 1/5000 dilution (purified) + Mouse spleen tissue lysate at 20 µg

Secondary
HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG at 1/50000 dilution

Predicted band size: 69 kDa
Observed band size: 72 kDa why is the actual band size different from the predicted?

Blocking and dilution buffer: 5% NFDM/TBST.

Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800) at 1/5000 dilution (purified) + A549 whole cell lysate at 20 µg

Secondary
HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG at 1/50000 dilution

Predicted band size: 69 kDa
Observed band size: 72 kDa why is the actual band size different from the predicted?

Blocking and dilution buffer: 5% NFDM/TBST.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat kidney tissue labelling COX2 / Cyclooxygenase 2 with purified ab179800 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. 

ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung carcinoma tissue labelling COX2 / Cyclooxygenase 2 with purified ab179800 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. 

ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.
Immunoprecipitation - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800) (purified) at 1/30 immunoprecipitating COX2 in A549 whole cell lysate.

Lane 1 (input): A549 whole cell lysate (10µg)

Lane 2 (+): ab179800 + A549 whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab179800 in A549 whole cell lysate.

For western blotting, HRP-conjugated anti-rabbit IgG, specific for the reduced form of IgG, was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.

Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800) at 1/1000 dilution (unpurified) + A549 cell lysate at 10 µg

**Predicted band size:** 69 kDa

Western blot analysis on immunoprecipitation pellet from A549 cell lysate using unpurified ab179800.
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