abcam

Product datasheet

Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] ab179800



Recombinant

RabMAb

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Overview

Product name Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012]

Description Rabbit monoclonal [EPR12012] to COX2 / Cyclooxygenase 2

Host species Rabbit

Specificity Stimulation is required to allow detection of the COX2 protein in some cell lines and tissues. It is

better to use a positive control side by side when testing.

Rat species is recommended based on IHC result, we do not guarantee WB, IP and ICC/IF for

Rat.

Tested applications Suitable for: WB, IP, ICC/IF, IHC-P

Unsuitable for: Flow Cyt

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: A549, U-87 MG and HeLa cell lysates; mouse spleen tissue lysate, PTGS2 (COX2 /

Cyclooxygenase 2) KO A549 (Human lung carcinoma epithelial cell) cell lysate, Wild-type A549 cell lysate, U-87 MG whole cell lysate, MCF7 whole cell lysate. Mouse B16-F10 and Raw 264.7 whole cell lysate. Mouse retina, hippocampus, heart and kidney tissue lysate. IHC-P: Human colonic carcinoma, lung carcinoma, liver and colon tissues: rat kidney tissue; mouse kidney and

liver tissue. IP: A549 cell lysate ICC: U-87 MG cells

General notesThis 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**.

Properties

Form Liquid

1

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

ClonalityMonoclonalClone numberEPR12012

Isotype IgG

Applications

The Abpromise guarantee 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.

Application	Abreviews	Notes
WB	★★★★ (1)	1/1000 - 1/5000. Predicted molecular weight: 69 kDa.
IP		1/10 - 1/100.
ICC/IF		Use at an assay dependent concentration.
IHC-P	★★★★★ (2)	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.

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 similaritiesBelongs to the prostaglandin G/H synthase family.

Contains 1 EGF-like domain.

Post-translational

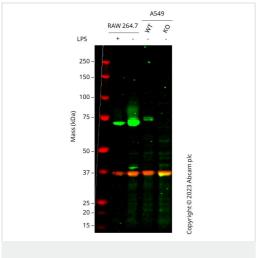
modifications

S-nitrosylation by NOS2 (iNOS) activates enzme activity. S-nitrosylation may take place on

different Cys residues in addition to Cys-561.

Cellular localization Microsome membrane. Endoplasmic reticulum membrane.

Images



Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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

Lane 1: RAW 264.7 Control LPS (0 ng/mL, 4 h) cell lysate

Lane 2: RAW 264.7 Treated LPS (100 ng/mL, 4 h) cell lysate

Lane 3: Wild-type A549 ab277305 cell lysate

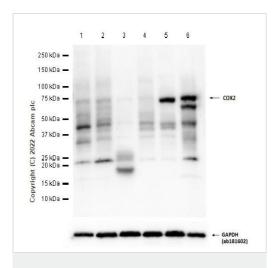
Lane 4: PTGS2 knockout A549 ab283802 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Western blot: Anti-PTGS2 antibody [EPR12012] (ab179800) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab179800 was shown to bind specifically to PTGS2. A band was observed at 69 kDa in wild-type RAW 264.7 cell lysates with no signal observed at this size in PTGS2 knockout cell line. To generate this image, wild-type and PTGS2 knockout RAW 264.7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% milk in TBS-0.1% Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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

Lane 1: B16-F10 (Mouse skin melanoma) whole cell lysate **Lane 2**: Raw 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate

Lane 3: Mouse retina tissue lysate

Lane 4: Mouse hippocampus tissue lysate

Lane 5 : Mouse heart tissue lysate

Lane 6 : Mouse kidney tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

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

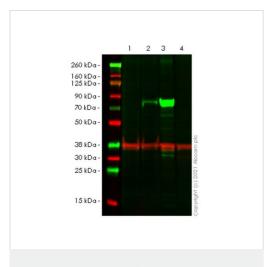
Exposure time: 60 seconds

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST

COX2 is expressed at a low level in Raw264.7, mouse retina, hippocampus, heart, kidney etc. (PMID: 22015457, PMID:

26001832, PMID: 23045674, PMID: 33737575).



Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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

Lane 1: PTGS2 (COX2 / Cyclooxygenase 2) KO A549 (Human lung carcinoma epithelial cell) cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

Lane 2 : Wild-type A549 cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

Lane 3 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell) whole cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

Lane 4: MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate with Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/100000 dilution

Performed under reducing conditions.

Predicted band size: 69 kDa **Observed band size:** 74 kDa

Negative control: MCF7 (PMID: 24325753, PMID: 16997132)

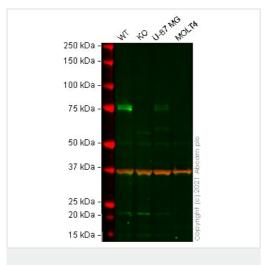
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

False colour image of Western blot: Anti- COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red.

In Western blot, ab179800 was shown to bind specifically to COX2 /

Cyclooxygenase 2. Target band was observed at 74 kDa in wild-type A549 cell lysates with no signal observed at this size in COX2 / Cyclooxygenase 2 knockout cell line ab280802. To generate this image, wild-type and COX2 / Cyclooxygenase 2 knockout A549 cell lysates were analyzed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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

Lane 1: Wild-type A549 cell lysate

Lane 2: PTGS2 knockout A549 cell lysate

Lane 3: U-87 MG cell lysate
Lane 4: MOLT-4 cell lysate

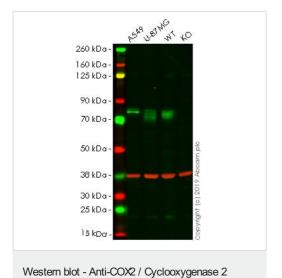
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 69 kDa

False colour image of Western blot: Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab179800 was shown to bind specifically to COX2 / Cyclooxygenase 2. A band was observed at 75 kDa in wild-type A549 cell lysates with no signal observed at this size in PTGS2 knockout cell line ab280802 (knockout cell lysate ab283825). To generate this image, wild-type and PTGS2 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C.

Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.



antibody [EPR12012] (ab179800)

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

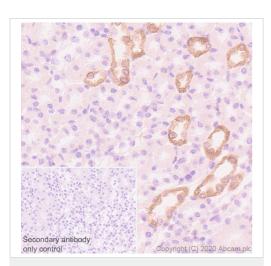
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 69 kDa

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

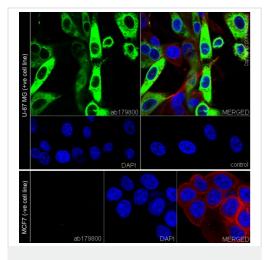
ab179800 was shown to react with COX2 / Cyclooxygenase 2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255420 (knockout cell lysate ab263795) was used. Wild-type and COX2 / Cyclooxygenase 2 knockout samples were subjected to SDS-PAGE. ab179800 and Anti-GAPDH antibody [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 lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG 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 paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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 μ g/ml).

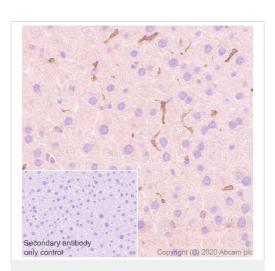
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>) was used as the secondary antibody. Negative control: secondary antibody only control. Hematoxylin was used as a counterstain.



Immunocytochemistry/ Immunofluorescence - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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.

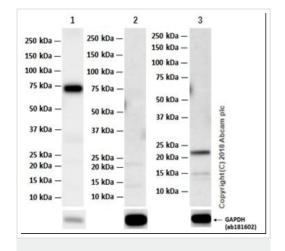
Confocal image showing cytoplasmic staining in U-87 MG cell line. Negative control: MCF7 \square PMID: 18199541 \square



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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 μ g/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 (<u>ab209101</u>) was used as the secondary antibody. Negative control: secondary antibody only control. Hematoxylin was used as a counterstain.



Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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 lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

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

Exposure time

Lane 1: 3.25 seconds

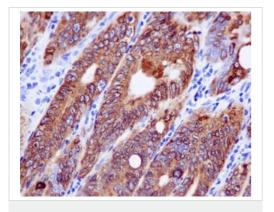
Lane 2 and 3: 180 seconds

The expression profile observed in HCT 116 and MCF7 are consistent with the literatures (PMID: 14739610, PMID: 24325753,

PMID: 16997132).

Negative control: HCT 116 (PMID: 14739610) and MCF7 (PMID:

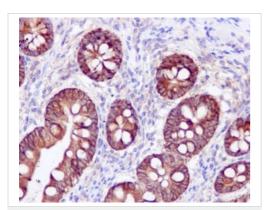
24325753, PMID: 16997132)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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.

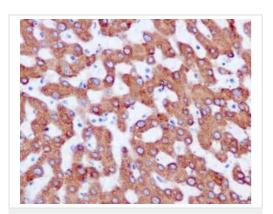
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded) analysis of human colon tissue labeling 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 paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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.



Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

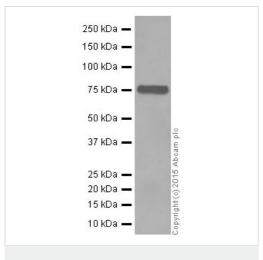
Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800) at 1/5000 dilution (purified) + Mouse spleen tissue lysate at 20 µg

Secondary

HRP-conjugated anti-rabbit $\lg G$, specific to the non-reduced form of $\lg G$ at 1/50000 dilution

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

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

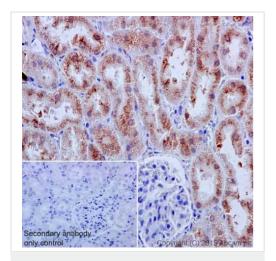
Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800) at 1/5000 dilution (purified) + A549 whole cell lysate at 20 µg

Secondary

HRP-conjugated anti-rabbit $\lg G$, specific to the non-reduced form of $\lg G$ at 1/50000 dilution

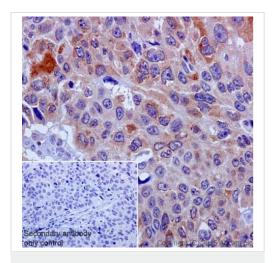
Predicted band size: 69 kDa **Observed band size:** 72 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



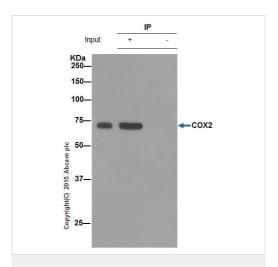
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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 paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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 lgG (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)

ab179800 (purified) at 1/30 immunoprecipitating COX2 in A549 whole cell lysate.

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

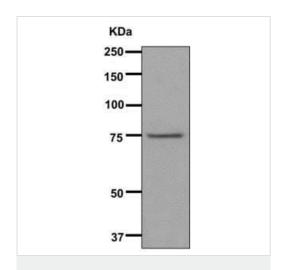
Lane 2 (+): ab179800 + A549 whole cell lysate.

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

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

Blocking buffer and concentration: 5% NFDM/TBST.

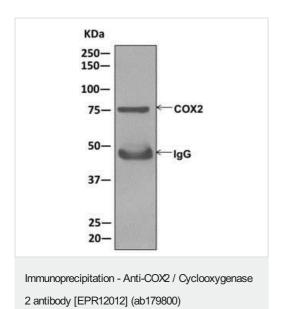
Diluting buffer and concentration: 5% NFDM /TBST.



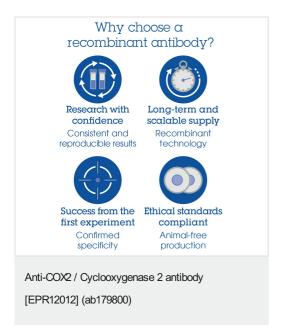
Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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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