abcam

Product datasheet

Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] ab179800





重组 RabMAb

★★★★★ 3 Abreviews 87 References 20 图像

概述

产品名称 Anti-COX2 / Cyclooxygenase 2抗体[EPR12012]

描述 兔单克隆抗体[EPR12012] to COX2 / Cyclooxygenase 2

宿主 Rabbit

特异性 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.

经测试应用 适用于: WB, IP, ICC/IF, IHC-P

不适用于: Flow Cyt

种属反应性 与反应: Mouse, Rat, Human

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

阳性对照 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

常规说明 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**.

性能

形式 Liquid

存放说明 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.

存储溶液 Preservative: 0.01% Sodium azide

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

纯**度** Protein A purified

克隆 单克隆

克隆编号 EPR12012

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab179800于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★ <u>(1)</u>	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 .

应用说明 Is unsuitable for Flow Cyt.

靶标

功能 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.

通路 Lipid metabolism; prostaglandin biosynthesis.

序列相似性 Belongs to the prostaglandin G/H synthase family.

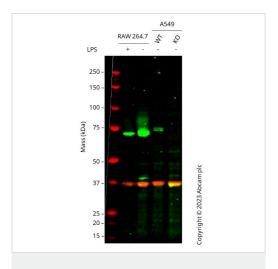
Contains 1 EGF-like domain.

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

different Cys residues in addition to Cys-561.

细胞定位 Microsome membrane. Endoplasmic reticulum membrane.

图片



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

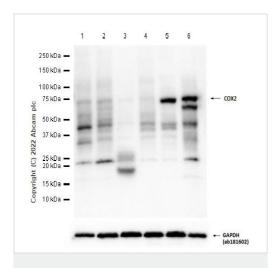
Lane 4: PTGS2 knockout A549 ab283802 cell lysate

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 virusinduced 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 lgG H&L (HRP) (ab97051) at 1/20000 dilution

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

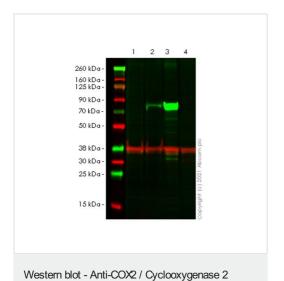
Exposure time: 60 seconds

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).



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

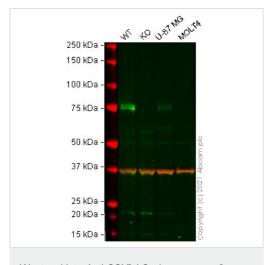
Negative control: MCF7 (PMID: 24325753, PMID: 16997132)

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 lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG 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

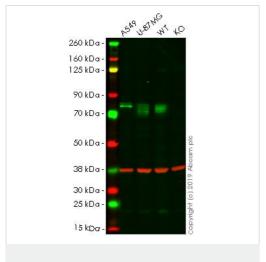
Lysates/proteins at 20 µg per lane.

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.



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.

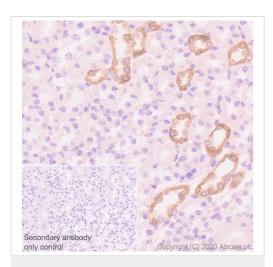
Performed under reducing conditions.

Predicted band size: 69 kDa

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

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

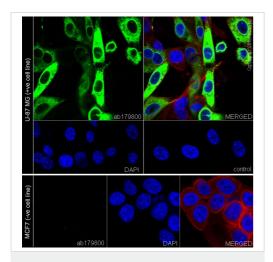
ab179800 was shown to react with COX2 / Cyclooxygenase 2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255420 (knockout cell lysate ab263795) was used. Wild-type and COX2 / Cyclooxygenase 2 knockout samples were subjected to SDS-PAGE. ab179800 and Anti-GAPDH antibody [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 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).

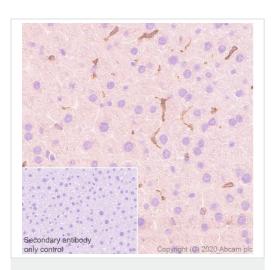
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: secondary antibody only control. Hematoxylin was used as a counterstain.



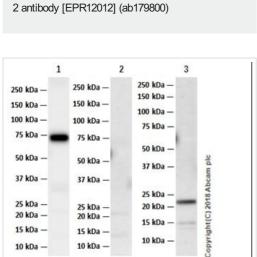
Immunocytochemistry/ Immunofluorescence - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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 (PMID: 18199541)



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



10 kDa

GAPDH (ab181602)

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

10 kDa -

10 kDa

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.

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

Exposure time

Lane 1: 3.25 seconds

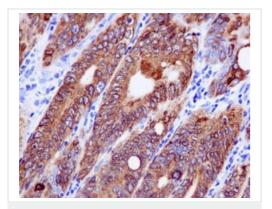
Lane 2 and 3: 180 seconds

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:

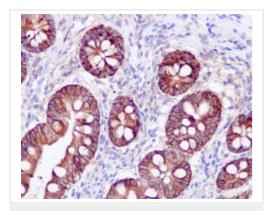
24325753, PMID: 16997132)



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.

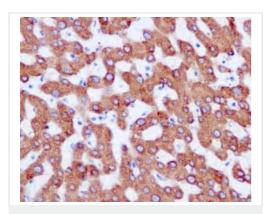
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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.

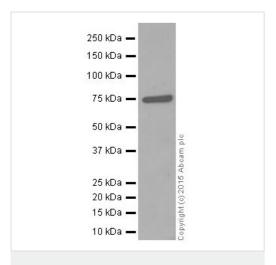
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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)

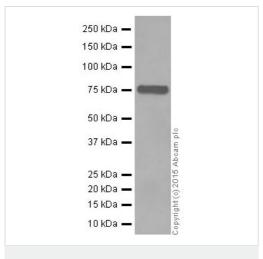
Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800) at 1/5000 dilution (purified) + Mouse spleen tissue lysate at 20 µg

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)

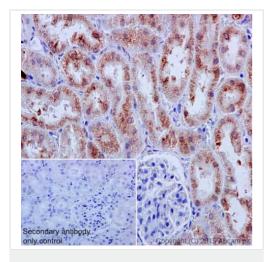
Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800) at 1/5000 dilution (purified) + A549 whole cell lysate at 20 µg

Secondary

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

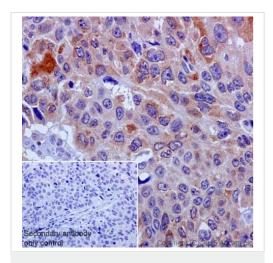
Predicted band size: 69 kDa **Observed band size:** 72 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



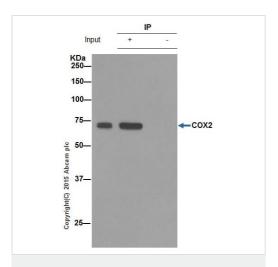
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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)

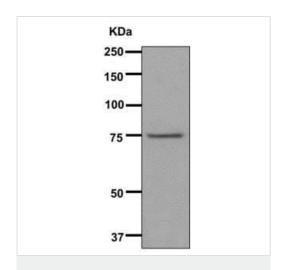
Lane 2 (+): ab179800 + A549 whole cell lysate.

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.

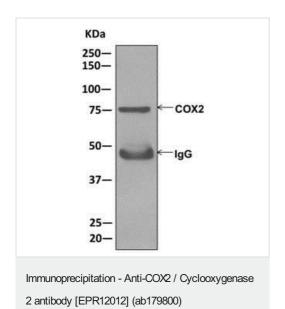
Diluting buffer and concentration: 5% NFDM /TBST.



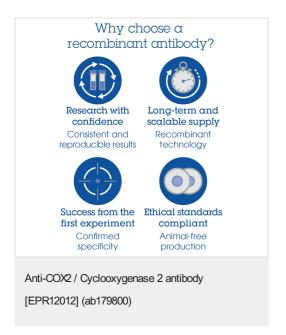
Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

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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