# abcam

#### Product datasheet

## Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free ab227528



★★★★★ 1 Abreviews 3 References

#### 製品の概要

製品名 Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free

製品の詳細 Rabbit monoclonal [EPR12012] to COX2 / Cyclooxygenase 2 - BSA and Azide free

由来種 Rabbit

特異性 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.

アプリケーション 適用あり: WB, IHC-P, IP, ICC/IF

適用なし: Flow Cyt

種交差性 交差種: Mouse, Rat, Human

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

ポジティブ・コントロール WB: A549, HeLa and U-87 cell lysates and mouse spleen tissue 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 and rat kidney tissue lysate;

mouse kidney and liver tissue. IP: A549 cell lysate.

特記事項 ab227528 is the carrier-free version of ab179800.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for

increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

#### 製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C. Do Not Freeze.

**バッファー** pH: 7.20

Constituent: PBS

キャリア・フリー はい

精製度 Protein A purified

**ポリモ**ノクローナル **ウローン名** EPR12012

アイソタイプ IgG

## アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab227528の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 69 kDa.
IHC-P	★★★☆☆ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.

追加情報 Is unsuitable for Flow Cyt.

#### ターゲット情報

機能 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.

パスウェイ Lipid metabolism; prostaglandin biosynthesis.

**配列類似性** Belongs to the prostaglandin G/H synthase family.

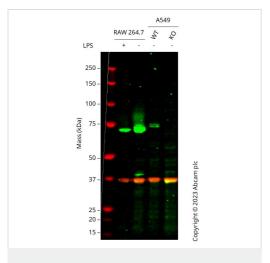
Contains 1 EGF-like domain.

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

different Cys residues in addition to Cys-561.

**細胞内局在** Microsome membrane. Endoplasmic reticulum membrane.

#### 画像



Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free (ab227528)

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

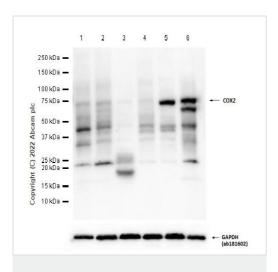
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (ab179800).

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] - BSA and Azide free (ab227528)

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 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

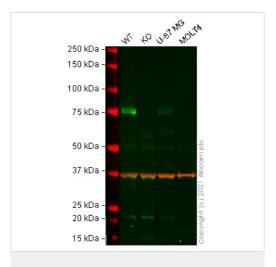
Blocking buffer and concentration:  $5\% \ NFDM/TBST$ 

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).

This data was developed using the same antibody clone in a different buffer formulation (<u>ab179800</u>).



Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free (ab227528)

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

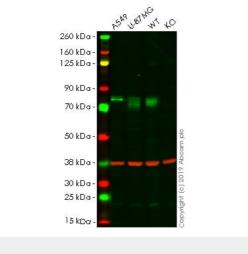
Lysates/proteins at 20 µg per lane.

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.

This data was developed using the same antibody clone in a different buffer formulation (**ab179800**).



Western blot - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free (ab227528)

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.

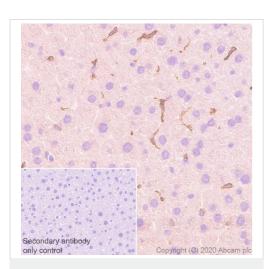
Performed under reducing conditions.

**Predicted band size:** 69 kDa **Observed band size:** 75 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab179800</u>).

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

<u>ab179800</u> was shown to react with COX2 / Cyclooxygenase 2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line <u>ab255420</u> (knockout cell lysate <u>ab263795</u>) was used. Wild-type and COX2 / Cyclooxygenase 2 knockout samples were subjected to SDS-PAGE. <u>ab179800</u> and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) 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<sup>®</sup> 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye<sup>®</sup> 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

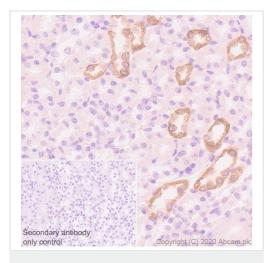


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free (ab227528)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue sections labeling COX2 / Cyclooxygenase 2 with purified  $\underline{ab179800}$  at 1/4000 dilution (0.125  $\mu$ 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab179800)

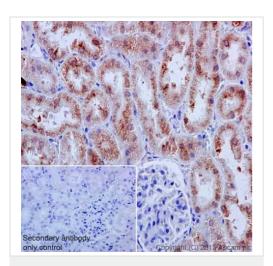


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free (ab227528)

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.

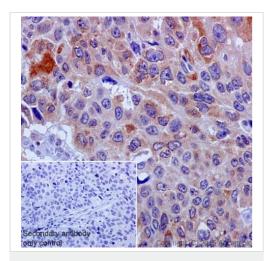
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab179800)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free (ab227528)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat kidney tissue labelling COX2 / Cyclooxygenase 2 with purified <a href="mailto:ab179800">ab179800</a> at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <a href="mailto:ab97051">ab97051</a>, 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.

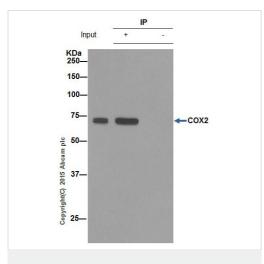
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab179800).



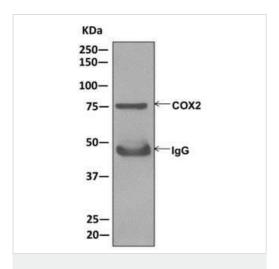
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free (ab227528)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung carcinoma tissue labelling COX2 / Cyclooxygenase 2 with purified <a href="mailto:ab179800">ab179800</a> at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <a href="mailto:ab97051">ab97051</a>, 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab179800).



Immunoprecipitation - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free (ab227528)



Immunoprecipitation - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free (ab227528)

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

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

Lane 2 (+): <u>ab179800</u> + A549 whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab179800</u> 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).

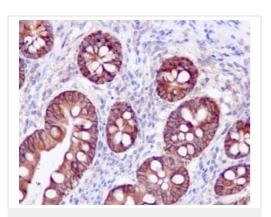
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab179800</u>).

Western blot analysis on immunoprecipitation pellet from A549 cell lysate using unpurified <u>ab179800</u>.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab179800</u>).

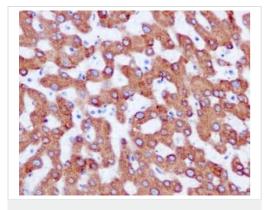


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free (ab227528)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded) analysis of human colon tissue labeling COX2 / Cyclooxygenase 2 with unpurified <u>ab179800</u> at a dilution of 1/250.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab179800).

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

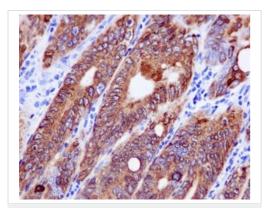


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free (ab227528)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded) analysis of human liver tissue labelling COX2 / Cyclooxygenase 2 with unpurified **ab179800** at a dilution of 1/250.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab179800).

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] - BSA and Azide free (ab227528)

This IHC data was generated using the same anti-COX2 / Cyclooxygenase 2 antibody clone, EPR12012, in a different buffer formulation (cat# <u>ab179800</u>).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colonic carcinoma tissue labelling COX2 with unpurified <u>ab179800</u> at a dilution of 1/250.

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



[EPR12012] - BSA and Azide free (ab227528)

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