abcam

Product datasheet

Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] ab180597



リコンピナント

RabMAb

6 References 画像数 14

製品の概要

製品名 Anti-Cytochrome P450 Reductase antibody [EPR14479(B)]

製品の詳細 Rabbit monoclonal [EPR14479(B)] to Cytochrome P450 Reductase

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt (Intra), WB, IHC-P, ICC/IF

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: HCT116, HEK-293T, HepG2, HeLa, MCF7 and A431 cell lysates, Mouse and Rat brain,

heart, spleen and kidney tissue lysates. ICC/IF: HeLa and MCF7 cells. IHC-P: Human infiltrating duct carcinoma of breast tissue, Mouse and Rat kidney tissue. Flow Cyt (intra): HeLa cells.

特記事項 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**.

製品の特性

製品の状態 Liquid

保存方法 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.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture

supernatant

精製度 Tissue culture supernatant

1

ポリ/モノ モノクローナル **ウローン名** EPR14479(B)

アイソタイプ lgG

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ab180597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
Flow Cyt (Intra)		1/10. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		1/10000 - 1/50000. Detects a band of approximately 77 kDa.
IHC-P		1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Heat up to 98 °C, below boiling, and then let cool for 10-20 min.
ICC/IF		1/500. For unpurified use at 1/100.

ターゲット情報

機能 This enzyme is required for electron transfer from NADP to cytochrome P450 in microsomes. It

can also provide electron transfer to heme oxygenase and cytochrome B5.

関連疾患 Defects in POR are the cause of adrenal hyperplasia variant type (AHV) [MIM:201750]; also

known as Antley-Bixler syndrome-like phenotype with disordered steroidogenesis. AHV is a rare variant of congenital adrenal hyperplasia. It is an autosomal recessive disorder with apparent combined P450C17 and P450C21 deficiency. Affected girls are born with ambiguous genitalia, but their circulating androgens are low and virilization does not progress. Conversely, affected boys are sometimes born undermasculinized. Boys and girls can also present with bone malformations, in some cases resembling the pattern seen in patients with Antley-Bixler

syndrome.

Defects in POR are a cause of isolated disordered steroidogenesis (IDS) [MIM:201750].

配列類似性 In the C-terminal section; belongs to the flavoprotein pyridine nucleotide cytochrome reductase

family.

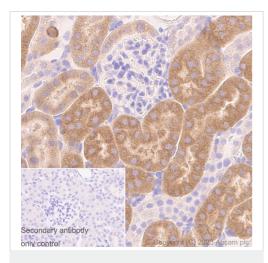
Contains 1 FAD-binding FR-type domain.

Contains 1 flavodoxin-like domain.

細胞内局在 Endoplasmic reticulum membrane. Anchored to the ER membrane by its N-terminal hydrophobic

region.

画像



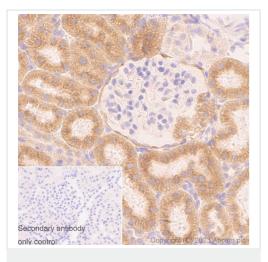
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytochrome P450
Reductase antibody [EPR14479(B)] (ab180597)

Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labelling Cytochrome P450 Reductase with ab180597 at 1/1000 (0.1 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). The section was counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

Postive staining on mouse kidney. The section was incubated with ab180597 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



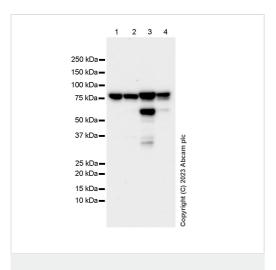
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytochrome P450
Reductase antibody [EPR14479(B)] (ab180597)

Immunohistochemical analysis of paraffin-embedded Rat kidney tissue labelling Cytochrome P450 Reductase with ab180597 at 1/1000 (0.1 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). The section was counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.

Postive staining on rat kidney. The section was incubated with ab180597 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument



Western blot - Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597)

All lanes : Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597) at 1/1000 dilution

Lane 1: Mouse brain tissue lysate

Lane 2: Mouse heart tissue lysate

Lane 3 : Mouse spleen tissue lysate

Lane 4: Mouse kidney tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/20000

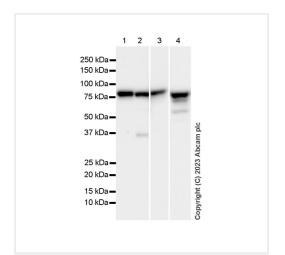
dilution

Observed band size: 75 kDa

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

Exposure time: Lane 1-3: 26 seconds; Lane 4-7: 75 seconds.

The identities of the lower MW bands between 37 and 60kDa are unknown.



Western blot - Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597)

All lanes : Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597) at 1/1000 dilution

Lane 1: Rat brain tissue lysate

Lane 2: Rat heart tissue lysate

Lane 3: Rat spleen tissue lysate

Lane 4: Rat kidney tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

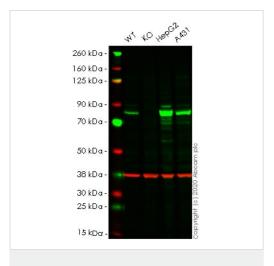
dilution

Observed band size: 75 kDa

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

Exposure time: Lane 1-3: 26 seconds; Lane 4-7: 75 seconds.

The identities of the lower MW bands between 37 and 60kDa are unknown.



Western blot - Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597)

All lanes : Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597) at 1/1000 dilution

Lane 1: Wild-type HCT116 cell lysate

Lane 2: POR knockout HCT116 cell lysate

Lane 3: HepG2 cell lysate

Lane 4: A431 cell lysate

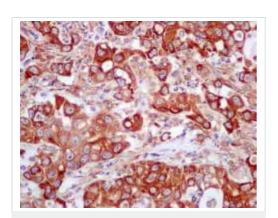
Lysates/proteins at 40 µg per lane.

Performed under reducing conditions.

Observed band size: 80 kDa

Lanes 1-4: Merged signal (red and green). Green - ab180597 observed at 80 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

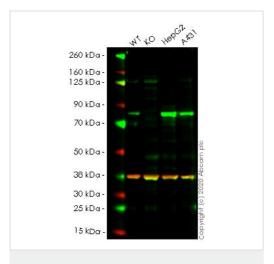
ab180597 was shown to react with Cytochrome P450 Reductase in wild-type HCT116 cells in western blot. Loss of signal was observed when knockout cell line ab266889 (knockout cell lysate ab257596) was used. Wild-type HCT116 and POR knockout HCT116 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab180597 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 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 paraffinembedded sections) - Anti-Cytochrome P450
Reductase antibody [EPR14479(B)] (ab180597)

Immunohistochemical analysis of paraffin embedded Human infiltrating duct carcinoma of breast tissue labeling Cytochrome P450 Reductase with ab180597 at a 1/150 dilution. HRP Polymer for Rabbit IgG secondary used. Counterstained with Hematoxylin.

Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



Western blot - Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597)

All lanes : Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597) at 1/10000 dilution

Lane 1: Wild-type HeLa lysate

Lane 2: Cytochrome P450 Reductase knockout HeLa lysate

Lane 3 : HepG2 lysate Lane 4 : A431 lysate

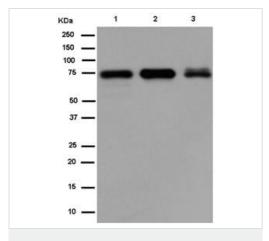
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Lanes 1-4: Merged signal (red and green). Green - ab180597 observed at 75 kDa. Red - loading control <u>ab8245</u> observed at 37 kDa.

ab180597 Anti-Cytochrome P450 Reductase antibody
[EPR14479(B)] was shown to specifically react with Cytochrome
P450 Reductase in wild-type HeLa cells. Loss of signal was
observed when knockout cell line <u>ab264996</u> (knockout cell lysate <u>ab257595</u>) was used. Wild-type and Cytochrome P450 Reductase knockout samples were subjected to SDS-PAGE. ab180597 and

Anti-GAPDH antibody [6C5] - Loading Control? (ab8245) were incubated overnight at 4°C at 1 in 10000 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.



Western blot - Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597)

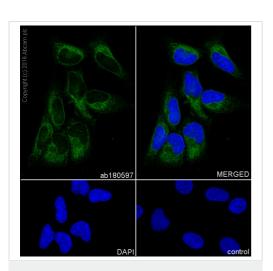
All lanes : Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597) at 1/10000 dilution

Lane 1 : HeLa lysate Lane 2 : MCF7 lysate Lane 3 : A431 lysate

Lysates/proteins at 20 µg per lane.

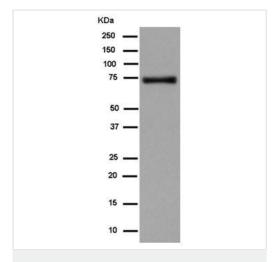
Secondary

All lanes : Goat Anti-Rabbit lgG (H+L) Peroxidase conjugated at 1/1000 dilution



Immunocytochemistry/ Immunofluorescence - Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597)

Immunocytochemistry/Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) labeling Cytochrome P450 Reductase with purified ab180597 at 1/500 dilution. Cells were fixed with 100% methanol. ab150077 Goat anti rabbit lgG (Alexa Fluor[®] 488) at 1/1000 was used as the secondary antibody. Nuclei were counterstained with DAPI. PBS was used instead of the primary antibody as the negative control.

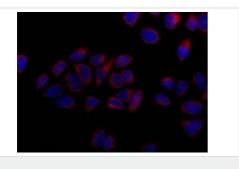


Western blot - Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597)

Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597) at 1/50000 dilution + HepG2 lysate at 20 μ g

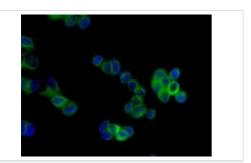
Secondary

Goat Anti-Rabbit lgG (H+L) Peroxidase conjugated at 1/1000 dilution



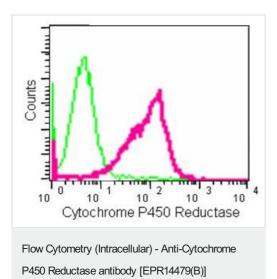
Immunocytochemistry/ Immunofluorescence - Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597)

Immunofluorescence analysis of Hela cells (fixative 4% paraformaldehyde) labeling Cytochrome P450 Reductase with ab180597 at a 1/100 dilution (red), and counterstained with Dapi (blue). Goat anti rabbit lgG (Dylight 555) secondary used at a 1/200 diution.



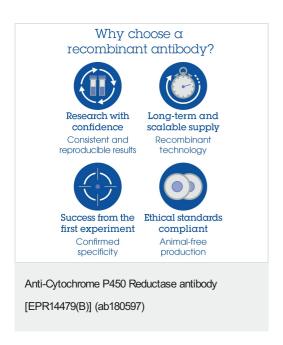
Immunocytochemistry/ Immunofluorescence - Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] (ab180597)

Immunofluorescence analysis of MCF7 cells (fixative 4% paraformaldehyde) labeling Cytochrome P450 Reductase with ab180597 at a 1/100 dilution (green), and counterstained with Dapi (blue). Goat anti rabbit lgG (Dylight 555) secondary used at a 1/200 diution.



(ab180597)

Intracellular Flow Cytometry analysis of HeLa cells using ab180597 at a 1/10 dilution (red) and a rabbit lgG as negative control (green). Goat anti rabbit lgG (FITC) secondary usedat a 1/150 dilution.



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