# abcam

## Product datasheet

## Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] -BSA and Azide free ab250241



יעלטעבע RabMAb

## 画像数7

### 製品の概要

製品名 Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] - BSA and Azide free

製品の詳細 Rabbit monoclonal [EPR14479(B)] to Cytochrome P450 Reductase - BSA and Azide free

由来種 Rabbit

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

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, HepG2, A431 and HeLa cell lysates, Mouse and Rat brain, heart, spleen and

kidney tissue lysates. IHC-P: Mouse and Rat kidney tissue.

特記事項 ab250241 is the carrier-free version of ab180597.

> 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.

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**.

1

#### 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: 7.2

Constituent: PBS

キャリア・フリー はい

精製度 Protein A purified

**ポリ/モノ** モノクローナル **クローン名** EPR14479(B)

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ab250241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)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 77 kDa (predicted molecular weight: 76 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.  For antigen retrieval, heat up to 98 degree C, below boiling, and then let cool for 10-20 minutes.

#### 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.

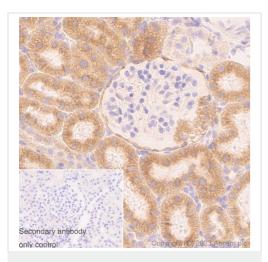
Contains 1 FAD-binding FR-type domain.

Contains 1 flavodoxin-like domain.

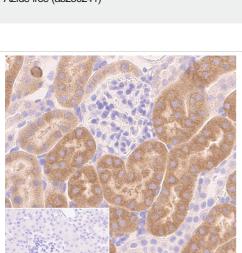
#### 細胞内局在

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)] - BSA and
Azide free (ab250241)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytochrome P450
Reductase antibody [EPR14479(B)] - BSA and
Azide free (ab250241)

This data was developed using the same antibody clone in a different buffer formulation (<u>ab180597</u>).

Immunohistochemical analysis of paraffin-embedded Rat kidney tissue labelling Cytochrome P450 Reductase with <u>ab180597</u> 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

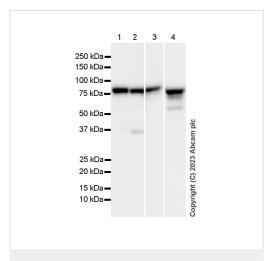
This data was developed using the same antibody clone in a different buffer formulation (ab180597).

Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labelling Cytochrome P450 Reductase with <u>ab180597</u> 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 <a href="mailto:ab180597"><u>ab180597</u></a> 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)] - BSA and Azide free (ab250241)

**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) (<u>ab97051</u>) at 1/20000 dilution

**Predicted band size:** 76 kDa **Observed band size:** 75 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab180597</u>).

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.

1 2 3 4

250 kDa—
150 kDa—
100 kDa—
75 kDa—
50 kDa—
37 kDa—
25 kDa—
20 kDa—
15 kDa—
10 kDa—
110 kDa—
10 kDa—

Western blot - Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] - BSA and Azide free (ab250241)

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

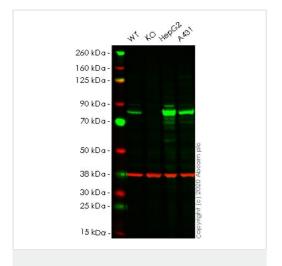
**Predicted band size:** 76 kDa **Observed band size:** 75 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab180597</u>).

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)] - BSA and Azide free (ab250241) **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.

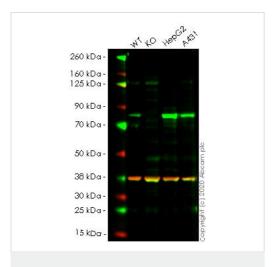
Predicted band size: 76 kDa
Observed band size: 80 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab180597</u>).

**Lanes 1-4:** Merged signal (red and green). Green - <u>ab180597</u> observed at 80 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) 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 lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD)

preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Cytochrome P450 Reductase antibody [EPR14479(B)] - BSA and Azide free (ab250241)

**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.

Predicted band size: 76 kDa

This data was developed using the same antibody clone in a different buffer formulation (ab180597).

**Lanes 1-4:** Merged signal (red and green). Green - <u>ab180597</u> 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 ab264996 (knockout cell lysate ab257595) 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 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.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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