abcam

Product datasheet

Anti-Cytochrome P450 3A4/CYP3A4 antibody [EPR6202] ab124921



Overview

Product name Anti-Cytochrome P450 3A4/CYP3A4 antibody [EPR6202]

Description Rabbit monoclonal [EPR6202] to Cytochrome P450 3A4/CYP3A4

Host species Rabbit

Specificity Cross-reactivity with other CYP3A family: ab124921 has high possibility of reacting with CYP3A7

based on our internal test.

Tested applications Suitable for: WB, IP, IHC-P

Species reactivity Reacts with: Human

Immunogen Synthetic peptide within Human Cytochrome P450 3A4/CYP3A4 aa 450-550 (C terminal). The

exact sequence is proprietary. (Peptide available as <u>ab234892</u>)

Positive control WB: Human fetal and liver cell lysates IHC-P: Human liver tissue IP: Human liver cell lysates

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 $^{\otimes}$ technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb^{\otimes} patents**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

Properties

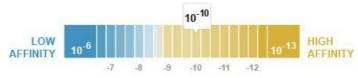
Form Liquid

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

Avoid freeze / thaw cycle.

Dissociation constant (K_D) $K_D = 2.63 \times 10^{-10} M$

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Learn more about K_D

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

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

Purity Protein A purified

ClonalityMonoclonalClone numberEPR6202

Isotype IgG

Applications

Target

The Abpromise guarantee Our Abpromise guarantee covers the use of ab124921 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. Predicted molecular weight: 57 kDa. For unpurified use at 1/10000 - 1/50000 dilution.
IP		1/10 - 1/100.
IHC-P		1/400. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. For unpurified use at 1/100 - 1/250 dilution. See IHC antigen retrieval protocols

Function	Cytochromes P450 are a group of heme-thiolate monooxygenases. In liver microsomes, this

enzyme is involved in an NADPH-dependent electron transport pathway. It performs a variety of oxidation reactions (e.g. caffeine 8-oxidation, omeprazole sulphoxidation, midazolam 1'-hydroxylation and midazolam 4-hydroxylation) of structurally unrelated compounds, including

steroids, fatty acids, and xenobiotics. The enzyme also hydroxylates etoposide.

Tissue specificity Expressed in prostate and liver. According to some authors, it is not expressed in brain

(PubMed:19094056). According to others, weak levels of expression are measured in some brain locations (PubMed:19359404 and PubMed:18545703). Also expressed in epithelium of the small intestine and large intestine, bile duct, nasal mucosa, kidney, adrenal cortex, epithelium of the gastric mucosa with intestinal metaplasia, gallbladder, intercalated ducts of the pancreas,

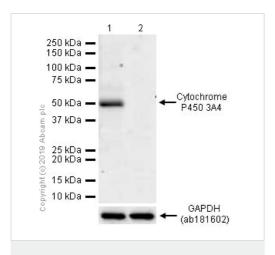
chief cells of the parathyroid and the corpus luteum of the ovary (at protein level).

Sequence similarities Belongs to the cytochrome P450 family.

Post-translational Polyubiquitinated in the presence of AMFR and UBE2G1 and also STUB1/CHIP and UBE2D1 (in

modifications vitro).

Images



Western blot - Anti-Cytochrome P450 3A4/CYP3A4 antibody [EPR6202] (ab124921)

All lanes : Anti-Cytochrome P450 3A4/CYP3A4 antibody [EPR6202] (ab124921) at 1/1000 dilution (Purified)

Lane 1: Human liver lysates

Lane 2 : HepG2 (Human hepatocellular carcinoma epithelial cell), Whole cell lysate

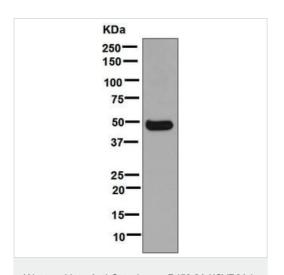
Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 57 kDa
Observed band size: 50 kDa

This antibody fails to detect BOI in normal HepG2 cells which is positive reported by PMID 26089940 and 25141173



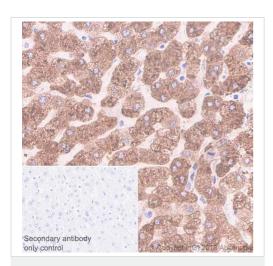
Western blot - Anti-Cytochrome P450 3A4/CYP3A4 antibody [EPR6202] (ab124921)

Anti-Cytochrome P450 3A4/CYP3A4 antibody [EPR6202] (ab124921) at 1/10000 dilution ((unpurified)) + Human fetal liver lysate at 10 µg

Secondary

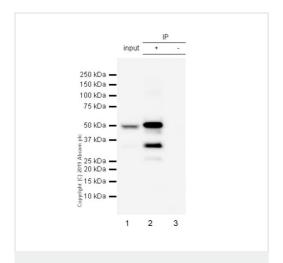
HRP labelled goat anti rabbit at 1/2000 dilution

Predicted band size: 57 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytochrome P450
3A4/CYP3A4 antibody [EPR6202] (ab124921)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human liver tissue sections labeling Cytochrome P450 3A4/CYP3A4 with purified ab124921 at 1/400 dilution (0.35 μg/ml). Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunoprecipitation - Anti-Cytochrome P450 3A4/CYP3A4 antibody [EPR6202] (ab124921)

ab124921 (purified) at 1/20 dilution (1ug) immunoprecipitating Cytochrome P450 3A4/CYP3A4 in Human liver lysates.

Lane 1: Human liver lysates 10ug

Lane 2 (+): ab124921 & Human liver lysates

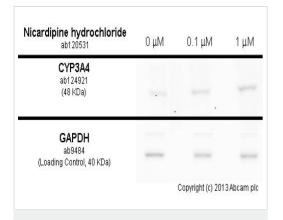
Lane 3 (-): Rabbit monoclonal IgG ($\underline{ab172730}$) instead of

ab124921 in Human liver lysates

For western blotting, VeriBlot for IP Detection Reagent (HRP)

(ab131366) was at 1/1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-Cytochrome P450 3A4/CYP3A4 antibody [EPR6202] (ab124921)

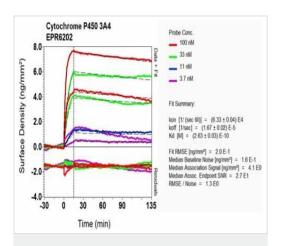
HepG2 cells were incubated at 37°C for 48h with vehicle control (0 μM) and different concentrations of nicardipine hydrochloride (<u>ab120531</u>) in DMSO. Increased expression of Cytochrome P450 3A4/CYP3A4 (ab124921, unpurified) correlates with an increase in nicardipine hydrochloride concentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 10 μ g of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 5% BSA before being incubated with ab124921 (unpurified) at 1/10000 dilution and <u>ab9484</u> at 1 μ g /ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP (<u>ab97051</u>) at 1/10000 dilution and visualised using ECL development solution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cytochrome P450
3A4/CYP3A4 antibody [EPR6202] (ab124921)

ab124921 (unpurified), at a 1/100 dilution, staining Cytochrome P450 3A4/CYP3A4 in paraffin embedded Human liver tissue by Immunohistochemistry.



OI-RD Scanning - Anti-Cytochrome P450 3A4/CYP3A4 antibody [EPR6202] (ab124921) Equilibrium disassociation constant (K_D) Learn more about K_D

Click here to learn more about KD



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