Datasheet for ABIN2777006

anti-CYP4B1 antibody (N-Term)

2 Validations

4 Images

Go to Product page

Overview

Quantity:	100 µL
Target:	CYP4B1
Binding Specificity:	N-Term
Reactivity:	Human, Mouse, Rat, Cow, Dog, Guinea Pig, Horse, Rabbit, Goat
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This CYP4B1 antibody is un-conjugated
Application:	Western Blotting (WB)

Product Details

Immunogen:	The immunogen is a synthetic peptide directed towards the N terminal region of human CYP4B1
Sequence:	SWAHQFPYAH PLWFGQFIGF LNIYEPDYAK AVYSRGDPKA PDVYDFFLQW
Predicted Reactivity:	Cow: 100%, Dog: 100%, Goat: 93%, Guinea Pig: 100%, Horse: 100%, Human: 100%, Mouse: 100%, Rabbit: 100%, Rat: 100%
Characteristics:	This is a rabbit polyclonal antibody against CYP4B1. It was validated on Western Blot using a cell lysate as a positive control.
Purification:	Affinity Purified

Target Details

Target:	CYP4B1

Target Details

Alternative Name:	CYP4B1 (CYP4B1 Products)
Background:	CYP4B1 is a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the endoplasmic reticulum. In rodents, the homologous protein has been shown to metabolize certain carcinogens, however, the specific function of the human protein has not been determined.This gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the endoplasmic reticulum. In rodents, the homologous protein has been shown to metabolize certain carcinogens, however, the specific function of the human protein has not been determined. Two transcript variants encoding slightly different isoforms have been found for this gene. Alias Symbols: CYPIVB1, P-450HP Protein Size: 511
Molecular Weight:	59 kDa
Gene ID:	1580
NCBI Accession:	NM_000779, NP_000770
UniProt:	P13584

Application Details

Application Notes:	Optimal working dilutions should be determined experimentally by the investigator.
Comment:	Antigen size: 511 AA
Restrictions:	For Research Use only

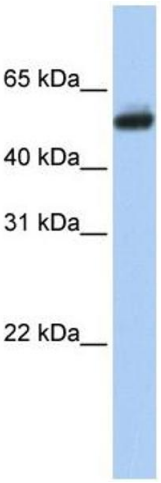
Handling

Format:	Liquid
Concentration:	Lot specific
Buffer:	Liquid. Purified antibody supplied in 1x PBS buffer with 0.09 % (w/v) sodium azide and 2 % sucrose.
Preservative:	Sodium azide

Handling

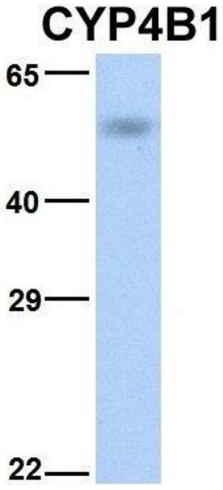
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	For short term use, store at 2-8°C up to 1 week. For long term storage, store at -20°C in small aliquots to prevent freeze-thaw cycles.

Images



Western Blotting

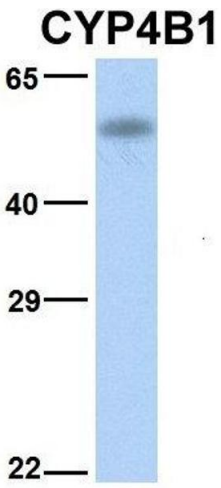
Image 1. WB Suggested Anti-CYP4B1 Antibody Titration: 0.2-1 ug/ml ELISA Titer: 1:312500 Positive Control: Human brain



Western Blotting

Image 2. Host: Rabbit Target Name: CYP4B1 Sample Type: Human Fetal Lung Antibody Dilution: 1.0ug/ml

Images



Western Blotting

Image 3. Host: Rabbit Target Name: CYP4B1 Sample Type: Human Fetal Liver Antibody Dilution: 1.0ug/ml

Please check the product details page for more images. Overall 4 images are available for ABIN2777006.

Validation report #100829 for Immunofluorescence (IF)

Successfully validated (Immunofluorescence (IF))

by Herz Zentrum Göttingen, Universitätsmedizin Göttingen

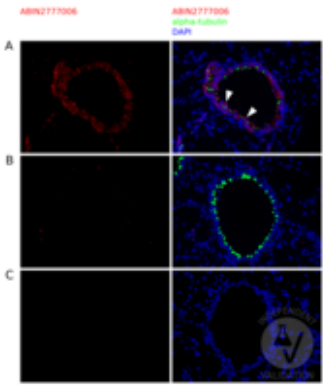
Report Number: 100829

Date: May 08 2017

Target:	CYP4B1
Lot Number:	QC12731-90422
Method validated:	Immunofluorescence (IF)
Positive Control:	WT lung small airway sections
Negative Control:	WT lung treated with naphthalene
Notes:	The antibody ABIN2777006 specifically stains CYP4B1 in fixed murine respiratory tissue.
Primary Antibody:	ABIN2777006
Secondary Antibody:	donkey anti-rabbit AF594 conjugated antibody (Invitrogen)

Protocol:	• Naphthalene treatment: i.p. application of 200mg per kg body weight (KG) naphthalene dissolved in oil.
	• Fix mouse lung tissue in 4% PFA /PBS ON at RT on a shaker.
	• Wash tissue 2-3x 60min with tap water/PBS.
	• Place tissue in
	  ◦ 70% EtOH 2x 15min or more
	  ◦ 80% EtOH 2x 30min
	  ◦ 90% EtOH 3x 30min
	  ◦ 100% ETOH 2x 90min
	  ◦ isopropanol ON
	  ◦ 25% Xylol/75% isopropanol 60min
	  ◦ 50% Xylol/50% isopropanol 60min
	  ◦ 75%Xylol/25% isopropanol 60min
	  ◦ xylol until clear (looks like jelly) ON
	  ◦ paraffin at 60°C for 3d changing every day
	• Embed tissue in paraffin and cool down to 4°C for a few minutes.
	• Cut blocks into 3µm sections using a semi-automated rotary microtome from Leica (RM2245).
	• Deparaffinize sections in:
	  ◦ xylol 2h
	  ◦ 50% xylol/50% isopropanol 10min
	  ◦ 100% isopropanol 10min

	○ 100% EtOH 5min ○ 90% EtOH 5min ○ 70% EtOH 5min • Wash sections briefly in distilled water. • Boil sections in 10mM citric acid buffer pH6.0 in a microwave for 20min. • Rinse sections in distilled water. • Wash sections 2x 5min in PBS. • Block sections in blocking solution (PBS, 0.05% Tween-20, 10% FCS) for 45min-60min at RT. • Wash sections 2x 5min in PBS. • Incubate sections with 70µl for each section primary ○ rabbit anti-cytochrome P450, family 4, subfamily B, polypeptide 1 (CYP4B1) (N-Term) antibody (antibodies-online, ABIN2777006, lot QC12731-90422) diluted 1:50 ○ mouse anti-alpha-tubulin antibody (Sigma, T 6793) diluted 1:1000 • in blocking solution for 16h at 4°C in a humidified chamber. • A no primary antibody negative control was incubated similarly in parallel. • Rinse sections 3x 5min with PBS. • Incubate sections with secondary ○ donkey anti-rabbit AF594 conjugated antibody (Invitrogen) ○ donkey anti-mouse AF488 conjugated antibody (Invitrogen) • diluted 1:500 in blocking solution for 1h at room temperature. • Wash sections 1x 5min with PBS containing 1:2000 diluted 1mg/ml DAPI stock solution. • Rinse sections 2x with PBS. • Mount slides with mounting medium (Dako). • Image acquisition with the same exposure and intensity for all IFs.
Experimental Notes:	• Staining of CYP4B1 with ABIN2777006 is visible in mouse WT lung small airway sections but not in negative control sections of naphthalene treated lungs in which club cells must have been depleted by apoptosis. • Club cells in the respiratory system are stained by ABIN2777006 in the smaller airways rather than big airways which is consistent with our literature knowledge. The Cyp450 antibody stains the non-ciliated cells, fitting the hypothesis perfectly. Pear-shaped stained cells reaching into the lumen of bronchiole also supports this finding.



Validation image no. 1 for anti-Cytochrome P450, Family 4, Subfamily B, Polypeptide 1 (CYP4B1) (N-Term) antibody (ABIN2777006)

Staining of mouse WT bronchioles with CYP4B1 antibody ABIN2777006 (red), DAPI (blue), and an acetylated alpha-tubulin specific antibody (cilia, green). CYP4B1 is detected in non-ciliated cell in untreated lung tissue (arrowheads) (A). No CYP4B1 staining of naphthalene treated samples (B) and the secondary antibody only negative control (C).

Validation report #101227 for Western Blotting (WB)

Successfully validated (Western Blotting (WB))

by Herz Zentrum Göttingen, Universitätsmedizin Göttingen

Report Number: 101227

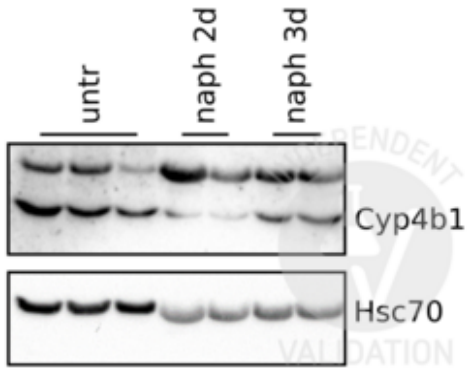
Date: May 08 2017

Target:	CYP4B1
Lot Number:	QC12731-90422
Method validated:	Western Blotting (WB)
Positive Control:	Lung lysates from untreated mice
Negative Control:	Lung lysates from naphthalene treated mice
Notes:	ABIN2777006 successfully reveals Cyp4b1 in murine respiratory tissue lysates.
Primary Antibody:	ABIN2777006
Secondary Antibody:	donkey anti-rabbit IgG (H+L) peroxidase-conjugated F(ab')2 fragment (Jackson ImmunoResearch)
Protocol:	• Naphthalene treatment: i.p. application of 200mg per kg body weight (KG) naphthalene dissolved in oil. • Denature 100µg total protein for 5min at 95°C in Laemmli SDS sample buffer and subsequently separate them on a denaturing gel SDS-PAGE gel in for 90min at 100V. • Transfer proteins onto 0.2µm nitrocellulose membrane in transfer buffer (15% MeOH, Tris 25mM, 86mM, SDS 70mM). • Block the membrane with blocking solution (PBS, 5% milk). • Incubation with primary ◦ rabbit anti-cytochrome P450, family 4, subfamily B, polypeptide 1 (CYP4B1) (N-Term) antibody (antibodies-online, ABIN2777006, lot QC12731-90422) diluted 1:300 or ◦ Hsc70 loading control antibody (supplier, product no, lot no) diluted • in blocking solution ON at 4°C. • Wash membrane 3x with blocking solution. • Incubate with secondary donkey anti-rabbit IgG (H+L) peroxidase-conjugated F(ab')2 fragment (Jackson ImmunoResearch) diluted1:10000 in blocking solution for 1h at RT. • Wash membrane 3x with PBS, 1x with blocking solution, 2x with PBS. • Develop membrane with SuperSignal West femto substrate (Thermo Fisher Scientific, 34095) according to manufacturer's recommendations, exposure time 25min. • Repeat the incubation steps with both antibodies, washes, and revealing the protein bands.
Experimental Notes:	• Staining of Cyp4b1 in murine lung lysates by ABIN2777006 was very weak with considerable

background after one incubation of the membranes with the antibody under the described conditions but did show a band of the expected MW at 59kDa. After a second round of incubation with both primary and secondary antibody this band was much stronger compared to the background.

- The 59kDa band is diminished in lysates taken two days after naphthalene treatment normalized to Hsc70. Its intensity increases again at day three.
- ABIN2777006 does also reveal a larger band than 59kDa which might represent ubiquitinylated or SUMOylated Cyp4b1. This would be consistent with this band being strongest 2d after the naphthalene treatment.

Image for Validation report #101227



Validation image no. 1 for anti-Cytochrome P450, Family 4, Subfamily B, Polypeptide 1 (CYP4B1) (N-Term) antibody (ABIN2777006)

Detection of Cyp4b1 by ABIN2777006 in untreated mouse respiratory tissue lysates from untreated mice (untr) and naphthalene treated mice 2d (naph 2d) and 3d (naph 3d) after the threatment.