

Anti-PBR antibody [EPR5384] ab109497

KO 評価済 リコンビナント RabMAb

★★★★★ [14 Abreviews](#) [97 References](#) [画像数 21](#)

製品の概要

製品名	Anti-PBR antibody [EPR5384]
製品の詳細	Rabbit monoclonal [EPR5384] to PBR
由来種	Rabbit
特異性	IHC results on rat tissues (such as liver and kidney) showed weak cytoplasmic and nuclear staining. However, other customer feedback suggests that this antibody works well in rat. Due to the inconclusive nature of these results, we do not currently guarantee this antibody in rat. Please contact our Scientific support team for more information.
アプリケーション	適用あり: IHC-Fr, Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF
種交差性	交差種: Mouse, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as ab170987)
ポジティブ・コントロール	WB: HCT116, HAP1, HeLa, U-87MG, 293T, A431, RAW264.7, and NIH3T3 cell lysates. Mouse lung, kidney, testis, brain, heart, liver and spleen tissue lysates. IHC-P: Human bladder carcinoma and colon tissue. Mouse lung, kidney, testis, brain, heart, liver and spleen tissue. IHC-Fr: Mouse kidney, adrenal gland tissue. ICC/IF: U-87MG, HeLa, MA-10 and TSPO-HAP1 cells; HeLa-TSPO (negative). Flow Cyt (intra): HepG2 and U-87MG cells. IP: A431 and U-87MG cell lysates.
特記事項	<p>The Peripheral-Type Benzodiazepine Receptor (PBR) is a protein which is primarily found on mitochondria, mediating cholesterol transport across the mitochondrial membranes in steroidogenic cells. PBR has been shown to play a regulatory role in tumor cell proliferation.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p> <p>Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.</p>

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. Avoid freeze / thaw cycle.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 59% PBS, 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR5384
アイソタイプ	IgG

アプリケーション

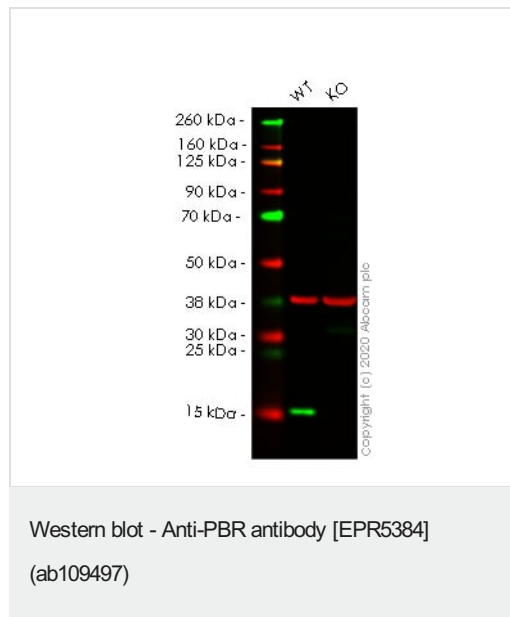
The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab109497の使用に適用されます**
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご確認ください。

アプリケーション	Abreviews	特記事項
IHC-Fr		1/250. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20)
Flow Cyt (Intra)		1/50 - 1/150. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (9)	1/10000 - 1/50000. Predicted molecular weight: 19 kDa.
IP		1/10 - 1/100.
IHC-P	★★★★★ (1)	1/100 - 1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols .
ICC/IF	★★★★★ (4)	Use a concentration of 1 µg/ml.

ターゲット情報

機能	Responsible for the manifestation of peripheral-type benzodiazepine recognition sites and is most likely to comprise binding domains for benzodiazepines and isoquinoline carboxamides. May play a role in the transport of porphyrins and heme. Plays a role in the transport of cholesterol across mitochondrial membranes in steroidogenic cells.
組織特異性	Found in many tissue types. Expressed at the highest levels under normal conditions in tissues that synthesize steroids.
配列類似性	Belongs to the TspO/BZRP family.

画像



All lanes : Anti-PBR antibody [EPR5384] (ab109497) at 1/1000 dilution

Lane 1 : Wild-type HCT116 cell lysate

Lane 2 : TSPO knockout HCT116 cell lysate

Lysates/proteins at 20 µg per lane.

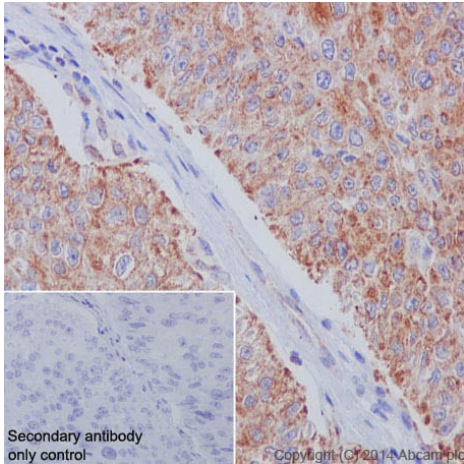
Performed under reducing conditions.

Predicted band size: 19 kDa

Observed band size: 17 kDa

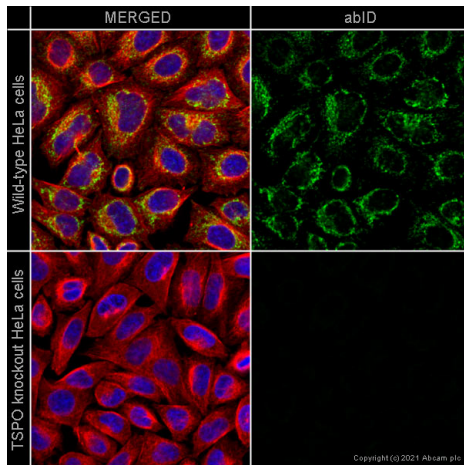
Lanes 1- 2: Merged signal (red and green). Green - ab109497 observed at 17 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

ab109497 was shown to react with PBR in wild-type HCT116 cells in western blot. Loss of signal was observed when knockout cell line [ab266878](#) (knockout cell lysate [ab257067](#)) was used. Wild-type HCT116 and TSPO 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. ab109497 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 paraffin-embedded sections) - Anti-PBR antibody [EPR5384] (ab109497)

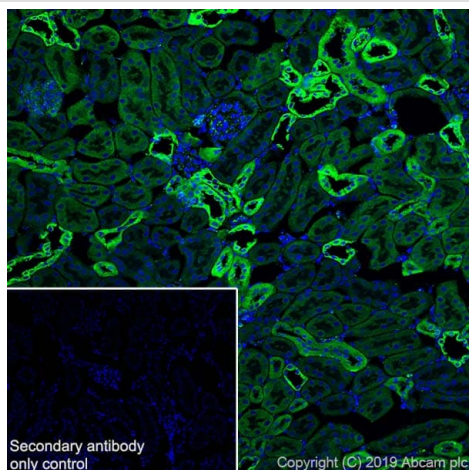
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human bladder carcinoma tissue labelling PBR with purified ab109497 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. [ab97051](#), a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-PBR antibody [EPR5384] (ab109497)

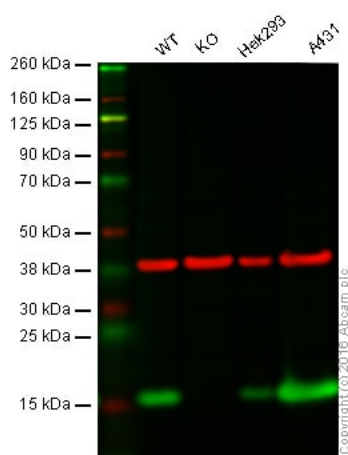
ab109497 staining PBR in wild-type HeLa cells (top panel) and TSPO knockout HeLa cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab109497 at 0.1 µg/ml and [ab195884](#) at 2 µg/ml (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) ([ab150081](#)) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Frozen sections) - Anti-PBR antibody [EPR5384] (ab109497)

Immunohistochemistry (Frozen sections) analysis of mouse kidney tissue sections labeling PBR with Purified ab109497 at 1/250 (3.8 µg/ml). Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20). Goat anti rabbit IgG (Alexa Fluor® 488, [ab150077](#)) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. DAPI was used as a counterstain.



Western blot - Anti-PBR antibody [EPR5384] (ab109497)

All lanes : Anti-PBR antibody [EPR5384] (ab109497) at 1/10000 dilution

Lane 1 : Wild-type HAP1 cell lysate

Lane 2 : PBR knockout HAP1 cell lysate

Lane 3 : HEK293 cell lysate

Lane 4 : A431 cell lysate

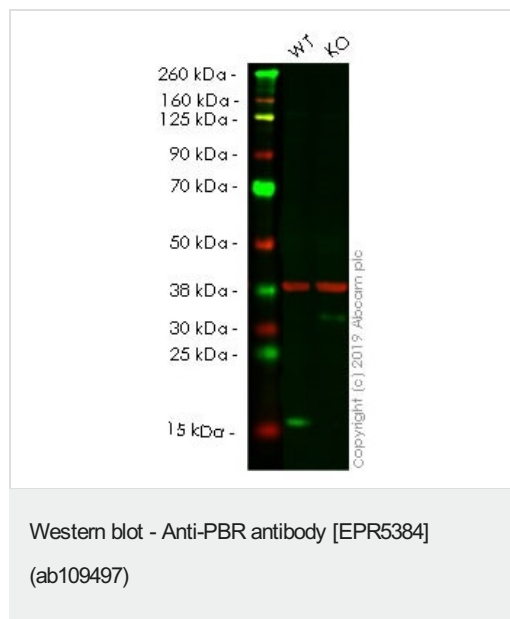
Lysates/proteins at 20 µg per lane.

Predicted band size: 19 kDa

Lanes 1 -4: Merged signal (red and green). Green - ab109497 observed at 15 kDa. Red - loading control, [ab8245](#), observed at 37 kDa.

ab109497 was shown to specifically react with PBR in wild type HAP1 cells. No band was observed when PBR knockout samples were used. Wild-type and PBR knockout samples were subjected to SDS-PAGE. Ab109497 and [ab8245](#) (loading control to GAPDH) were diluted at 1/10000 and 1/10000 dilution respectively and incubated overnight at 4C. 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 1/10000 dilution for 1 hour at room temperature before imaging.



All lanes : Anti-PBR antibody [EPR5384] (ab109497) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : TSPO (PBR) knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

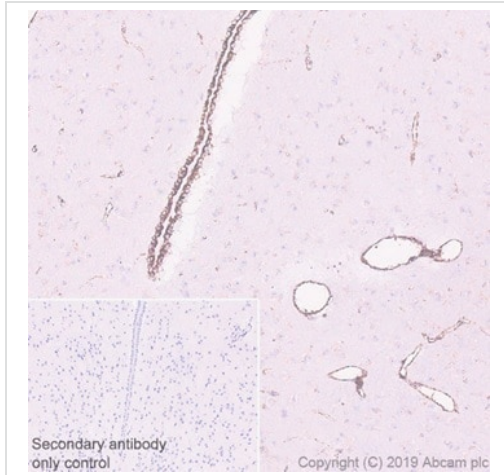
Performed under reducing conditions.

Predicted band size: 19 kDa

Observed band size: 15 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab109497 observed at 15 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

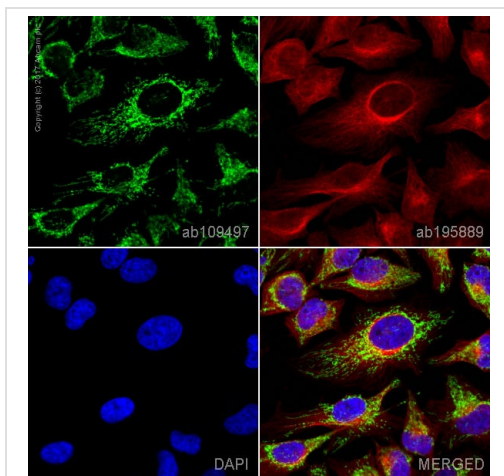
ab109497 was shown to react with PBR in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab264942** (knockout cell lysate **ab257066**) was used. Wild-type HeLa and TSPO knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab109497 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 paraffin-embedded sections) - Anti-PBR antibody [EPR5384] (ab109497)

Immunohistochemistry (formalin/PFA-fixed paraffin-embedded sections) analysis of mouse hypothalamus tissue labelling PBR with ab109497 at 1/1000 dilution (1.03 µg/ml). Heat mediated antigen retrieval was performed using Tris/EDTA buffer, pH 9.0 ([ab93684](#)). A goat anti-rabbit IgG (H+L) (HRP) was used as the secondary antibody (concentration: ready to use). A secondary-antibody-only control was also performed. Counterstained with hematoxylin.

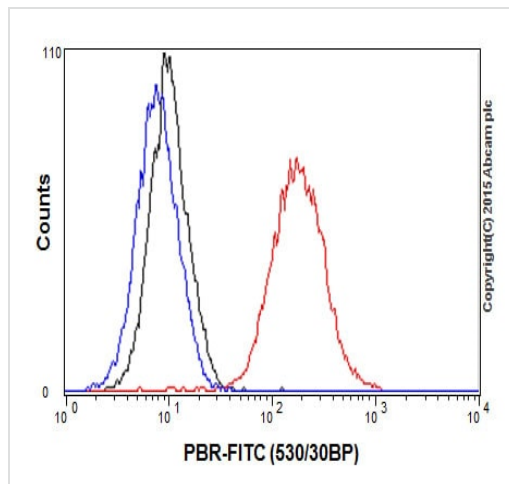
Positive staining was observed on ependymal cells and endothelial cells in mouse hypothalamus.



Immunocytochemistry/ Immunofluorescence - Anti-PBR antibody [EPR5384] (ab109497)

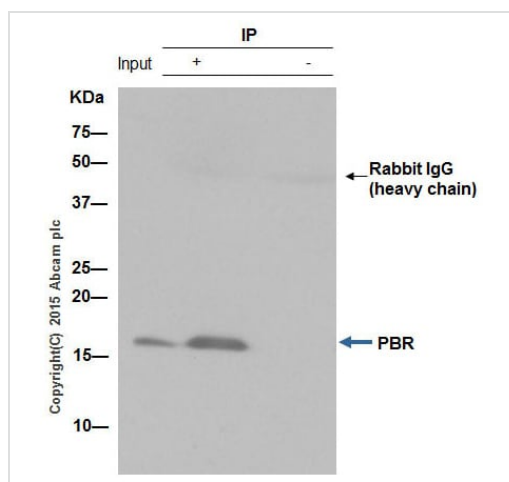
ab109497 staining PBR in HeLa. The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab109497 at 1µg/ml and [ab195889](#) at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) ([ab150081](#)) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Flow Cytometry (Intracellular) - Anti-PBR antibody
[EPR5384] (ab109497)

Intracellular Flow Cytometry analysis of U-87MG cells labelling PBR with purified ab109497 at 1/150 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Immunoprecipitation - Anti-PBR antibody [EPR5384]
(ab109497)

ab109497 (purified) at 1/60 immunoprecipitating PBR in A431 whole cell lysate.

Lane 1 (input): A431 whole cell lysate (10µg)

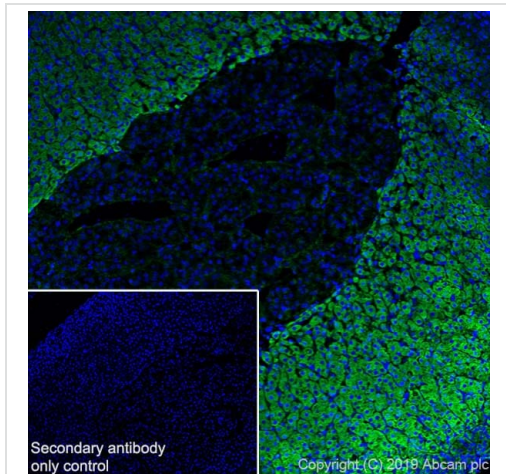
Lane 2 (+): ab109497 + A431 whole cell lysate (10µg).

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab109497 in A431 whole cell lysate.

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1500 dilution.

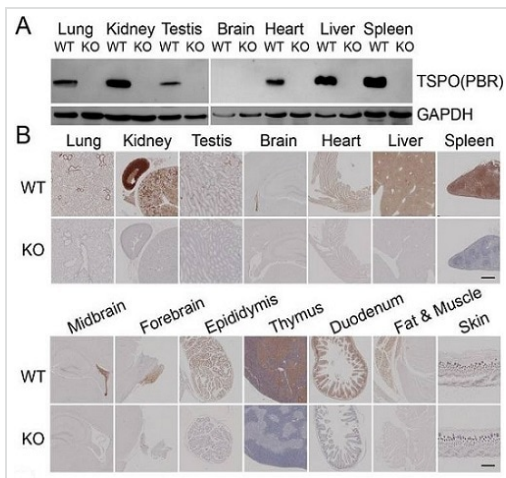
Blocking buffer and concentration: 5% NFDm/TBST.

Diluting buffer and concentration: 5% NFDm /TBST.



Immunohistochemistry (Frozen sections) - Anti-PBR antibody [EPR5384] (ab109497)

Immunohistochemistry (Frozen sections) analysis of mouse adrenal gland tissue sections labeling PBR with Purified ab109497 at 1/250 (3.8 µg/ml). Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. DAPI was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PBR antibody [EPR5384] (ab109497)

Wang, H. et al PLoS One. 2016 Dec 1;11(12):e0167307. doi: 10.1371/journal.pone.0167307. eCollection 2016
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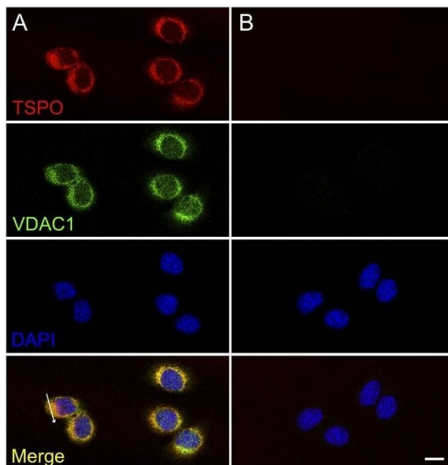
TSPO (PBR) expression was abolished in global KO mice without pathological changes

TSPO expression in different tissues from WT and KO mice was detected by western blotting (A) and IHC (B). Scale Bars, 100µm.

4% PFA-fixed tissue sections were blocked with 5% goat serum and incubated overnight at 4°C with ab109497 at 1/1500 dilution. DAB staining.

Note: TSPO and PBR are alternative names for the same target.

(Adapted from Figure 3 of Wang et al)



Immunocytochemistry/ Immunofluorescence - Anti-PBR antibody [EPR5384] (ab109497)

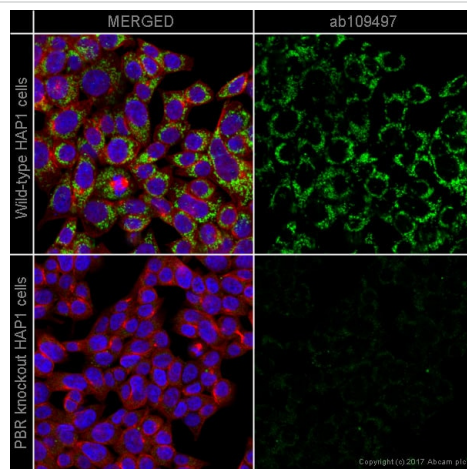
Morohaku, K. et al Send to PLoS One. 2013 Sep 5;8(9):e74509. doi: 10.1371/journal.pone.0074509. eCollection 2013 Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>

TSPO (PBR) expression is localized to the mitochondria

(A) Panel shows confocal images of TSPO (red), VDAC1 (green) and nuclear counterstain (blue) in MA-10 Leydig (mouse). cells. (B) Negative control panel. Colocalization of TSPO to the mitochondrial protein VDAC1 validates the specific localization of TSPO. Scale bar 20 μ m.

Cells were fixed with 4% formaldehyde and permeabilized using 0.1% Triton X-100. Cells were then blocked using 5% normal goat serum and incubated with ab109497 at 1/200 dilution.

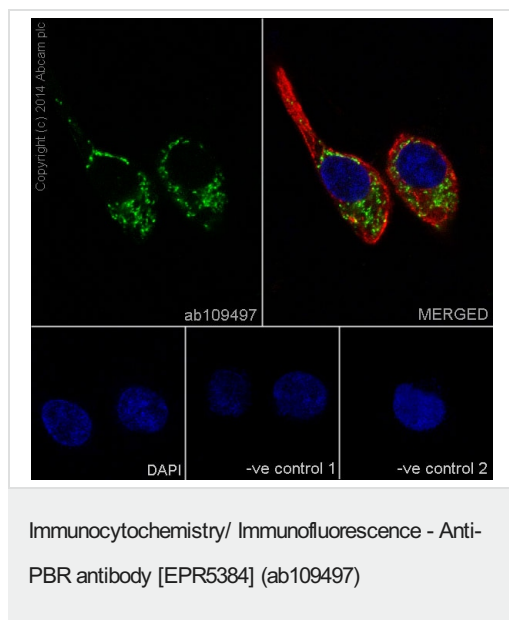
Note: TSPO and PBR are alternative names for the same target.



Immunocytochemistry/ Immunofluorescence - Anti-PBR antibody [EPR5384] (ab109497)

ab109497 staining PBR in wild-type HAP1 cells (top panel) and PBR knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab109497 at 1 μ g/ml and **ab195889** at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 μ g/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

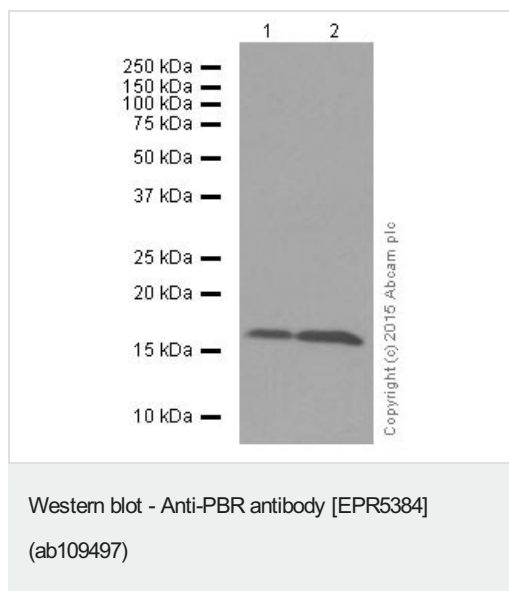
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/Immunofluorescence analysis of U-87MG cells labelling PBR with purified ab109497 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500) were also used.

Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).



All lanes : Anti-PBR antibody [EPR5384] (ab109497) at 1/50000 dilution (purified)

Lane 1 : RAW264.7 cell lysate

Lane 2 : NIH/3T3 cell lysate

Lysates/proteins at 20 µg per lane.

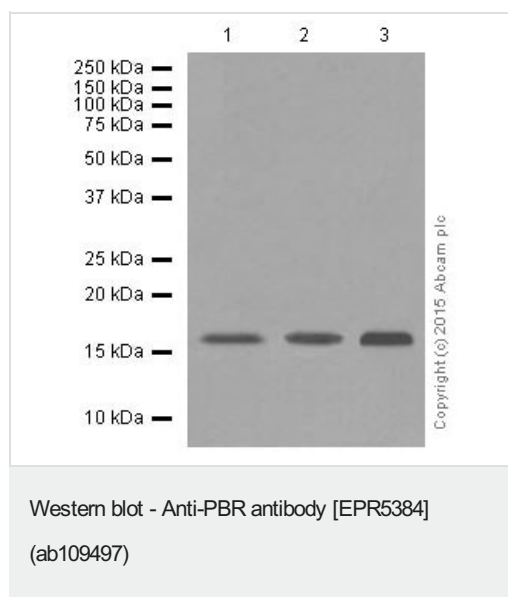
Secondary

All lanes : Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 19 kDa

Observed band size: 18 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



All lanes : Anti-PBR antibody [EPR5384] (ab109497) at 1/50000 dilution (purified)

Lane 1 : U-87MG cell lysate

Lane 2 : HEK293 cell lysate

Lane 3 : A431 cell lysate

Lysates/proteins at 20 µg per lane.

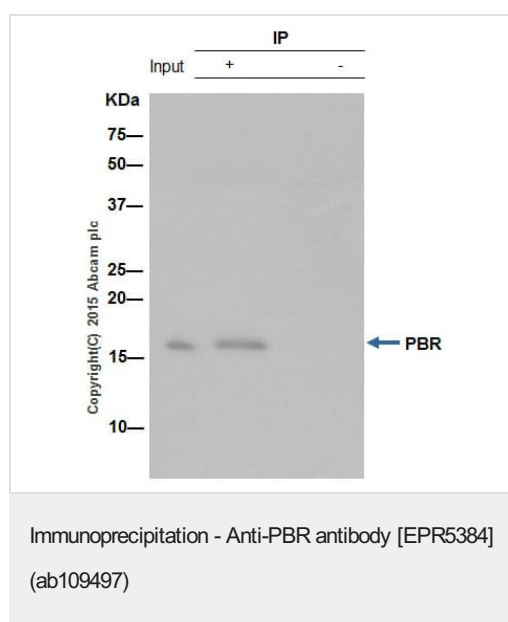
Secondary

All lanes : Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 19 kDa

Observed band size: 18 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



ab109497 (purified) at 1/60 immunoprecipitating PBR in U-87MG whole cell lysate.

Lane 1 (input): U-87MG whole cell lysate (10µg)

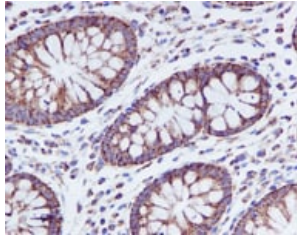
Lane 2 (+): ab109497 + U-87MG whole cell lysate (10µg).

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab109497 in U87-MG whole cell lysate.

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1500 dilution.

Blocking buffer and concentration: 5% NFDM/TBST.

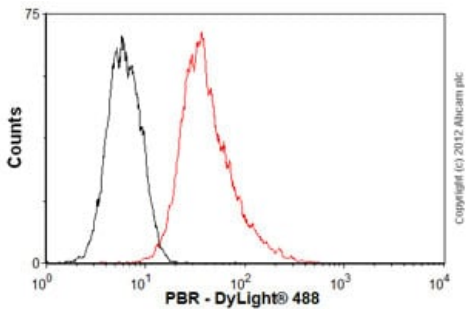
Diluting buffer and concentration: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PBR antibody [EPR5384] (ab109497)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon tissue labelling PBR with unpurified ab109497 at a dilution of 1/100.

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



Flow Cytometry (Intracellular) - Anti-PBR antibody [EPR5384] (ab109497)

Overlay histogram showing HepG2 cells stained with unpurified ab109497 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (unpurified ab109497, 1/50 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) ([ab96899](#)) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.

Why choose a recombinant antibody?



Anti-PBR antibody [EPR5384] (ab109497)

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