

Product datasheet

Anti-Heme Oxygenase 1 antibody [EPR18161-128] ab189491

KO VALIDATED Recombinant RobMAb

Properties

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Overview		
Product name	Anti-Heme Oxygenase 1 antibody [EPR18161-128]	
Description	Rabbit monoclonal [EPR18161-128] to Heme Oxygenase 1	
Host species	Rabbit	
Tested applications	Suitable for: WB, IHC-P, IP, ICC/IF, Flow Cyt (Intra)	
Species reactivity	Reacts with: Mouse, Rat, Human	
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.	
Positive control	WB: Human, mouse and rat spleen lysate. NIH/3T3 and HeLa cell lysate. IHC-P: Human, mouse and rat liver tissue. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt (intra): HeLa and NIH/3T3 cells. IP: NIH/3T3 whole cell lysate.	
General notes	 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 <u>see here</u>. Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>. 	

Topenies	
Form	Liquid
Storage instructions	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.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR18161-128
lsotype	lgG

Applications

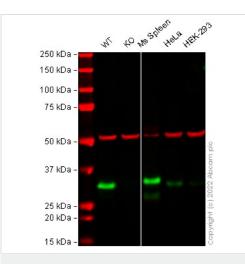
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab189491 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ (1)	1/2000. Predicted molecular weight: 33 kDa.
ІНС-Р		1/20000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.
ICC/IF	★★★★★(1)	1/250.
Flow Cyt (Intra)		1/50.

Target	
Function	Heme oxygenase cleaves the heme ring at the alpha methene bridge to form biliverdin. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. Under physiological conditions, the activity of heme oxygenase is highest in the spleen, where senescent erythrocytes are sequestrated and destroyed.
Sequence similarities	Belongs to the heme oxygenase family.
Cellular localization	Microsome. Endoplasmic reticulum.

Images



Western blot - Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491) All lanes : Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491) at 1/2000 dilution

Lane 1 : Wild-type A549 cell lysate Lane 2 : HMOX1 knockout A549 cell lysate Lane 3 : Mouse Spleen cell lysate Lane 4 : HeLa cell lysate Lane 5 : HEK-293 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

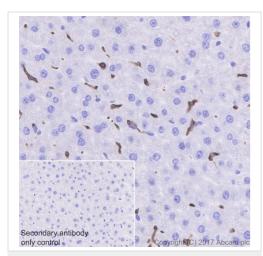
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 33 kDa Observed band size: 32 kDa

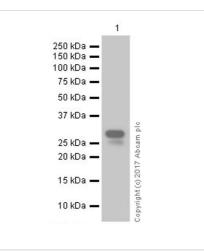
False colour image of Western blot: Anti-Heme Oxygenase 1 antibody [EPR18161-128] staining at 1/2000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab189491 was shown to bind specifically to Heme Oxygenase 1. A band was observed at 32 kDa in wild-type A549 cell lysates with no signal observed at this size in HMOX1 knockout cell line **ab269503** (HMOX knockout A549 lysate **ab259782**).

To generate this image, wild-type and HMOX1 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween®20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit lgG H&L 800CW and Goat anti-Mouse lgG H&L 680RD at 1/20000 dilution



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491) Immunohistochemical analysis of paraffin embedded mouse liver tissue labeling Heme Oxygenase 1 with ab189491 at 1/20000 dilution, followed by a ready to use Goat Anti-Rabbit lgG H&L (HRP). Positive staining on Kupffer cells of mouse liver (PMID: 9449694) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Western blot - Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491) Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491) at 1/2000 dilution + Human spleen lysate at 10 µg

Secondary

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/4000 dilution

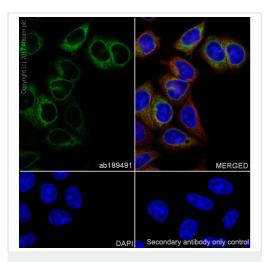
Predicted band size: 33 kDa Observed band size: 28, 32 kDa

Exposure time: 3 minutes

Blocking: 5% NFDM/TBST.

The molecular weight observed is consistent with what has been described in the literature (PMID: 18400743).

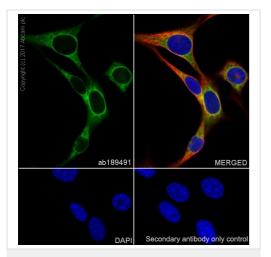
The lower band observed is a truncated form of Heme Oxygenase 1 (PMID: 17430897).



Immunocytochemistry/ Immunofluorescence - Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491)

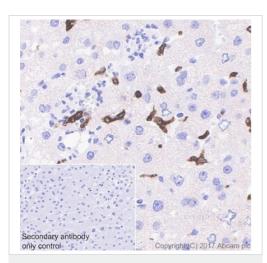
Immunofluorescent analysis of 4% paraformaldehyde fixed, 0.1% Triton X-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labeling Heme Oxygenase 1 with ab189491 at 1/250 dilution, followed by **ab150077** AlexaFluor[®]488 Goat anti-Rabbit secondary at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on HeLa cells. Details of counterstains: **ab195889** Anti-alpha Tubulin antibody [DM1A] -Microtubule Marker (Alexa Fluor[®] 594) at a 1/200 dilution; DAPI for nuclei.

The negative controls are as follows: Secondary antibody only for control.



Immunocytochemistry/ Immunofluorescence - Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491) Immunofluorescent analysis of 4% paraformaldehyde fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labeling Heme Oxygenase 1 with ab189491 at 1/250 dilution, followed by **ab150077** AlexaFluor[®]488 Goat anti-Rabbit secondary at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on NIH/3T3 cells. Details of counterstains: **ab195889** Antialpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) at a 1/200 dilution; DAPI for nuclei.

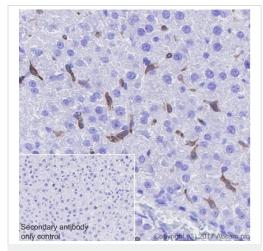
The negative controls are as follows: Secondary antibody only for control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491)

Immunohistochemical analysis of paraffin embedded human liver tissue labeling Heme Oxygenase 1 with ab189491 at 1/20,000 dilution, followed by a ready to use Goat Anti-Rabbit lgG H&L (HRP). Positive staining on Kupffer cells of human liver (PMID: 9449694) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP).

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).

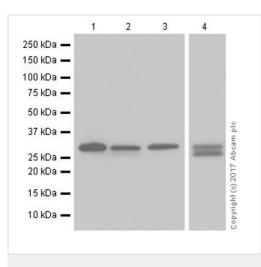


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491)

Immunohistochemical analysis of paraffin embedded rat liver tissue labeling Heme Oxygenase 1 with ab189491 at 1/20,000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining on Kupffer cells of rat liver (PMID: 9449694) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary

antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Western blot - Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491) All lanes : Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491) at 1/5000 dilution

Lane 1 : NIH/3T3 (mouse embryonic fibroblast) lysate
Lane 2 : Rat spleen lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell) lysate
Lane 4 : Mouse spleen lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 33 kDa Observed band size: 28, 32 kDa

Blocking: 5% NFDM/TBST.

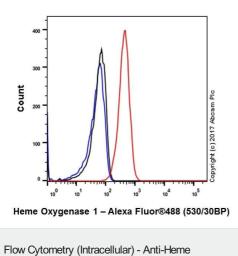
Exposure time:

Lanes 1,2 and 3: 2 seconds;

Lane 4: 1 second

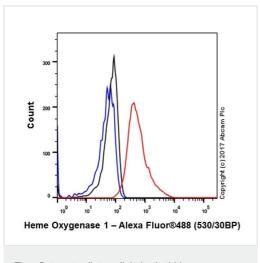
The molecular weight observed is consistent with what has been described in the literature (PMID: 18400743).

The lower band observed is a truncated form of Heme Oxygenase 1 (PMID: 17430897).

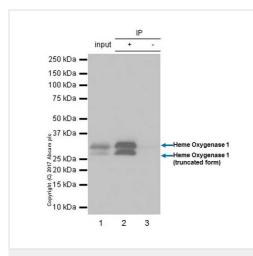


Intracellular flow cytometric analysis of 90% methanol/ 4% paraformaldehyde fixed NIH/3T3 (mouse embryonic fibroblast) cell line labeling Heme Oxygenase1 with ab189491 at 1/50 (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluorr[®] 488, **ab150077**), at 1/2000 dilution was used as the secondary antibody.

Flow Cytometry (Intracellular) - Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491)



Flow Cytometry (Intracellular) - Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491)



Immunoprecipitation - Anti-Heme Oxygenase 1 antibody [EPR18161-128] (ab189491) Intracellular flow cytometric analysis of 90% methanol/4% paraformaldehyde fixed HeLa (human cervix adenocarcinoma epithelial cell) cell line labeling Heme Oxygenase1 with ab189491 at 1/50 (Red) compared with a Rabbit monoclonal IgG (<u>ab172730</u>) (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluorr[®] 488, <u>ab150077</u>), at 1/2000 dilution was used as the secondary antibody.

Heme Oxygenase 1 was immunoprecipitated from 0.35 mg of NIH/3T3 (mouse embryonic fibroblast) whole cell lysate with ab189491 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab189491 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution.

Lane 1: NIH/3T3 (mouse embryonic fibroblast) whole cell lysate 10 μ g (Input).

Lane 2: NIH/3T3 whole cell lysate (+).

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab189491 in NIH/3T3 whole cell lysate (-).

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

The truncated form of Heme Oxygenase 1 is described in the literature (PMID: 17430897).



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