


Product datasheet

Anti-Heme Oxygenase 1 antibody [EP1391Y] ab52947

KO VALIDATED Recombinant RabMAB

★★★★★ [5 Abreviews](#) [82 References](#) [11 Images](#)

Overview

Product name	Anti-Heme Oxygenase 1 antibody [EP1391Y]
Description	Rabbit monoclonal [EP1391Y] to Heme Oxygenase 1
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), WB, IHC-P, IP
Species reactivity	Reacts with: Mouse, Human Predicted to work with: Guinea pig 
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Fetal liver lysate, human liver tissue, A549, HL-60, MCF7, NIH/3T3, HEK-293, and HeLa cells. IHC-P: FFPE mouse spleen normal. Human liver and human and mouse spleen tissue. Human spleen tissue. IP: Mouse spleen tissue lysate Flow Cyt (intra): NIH/3T3 cells.
General notes	<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>

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. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1391Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab52947 in the following tested applications.

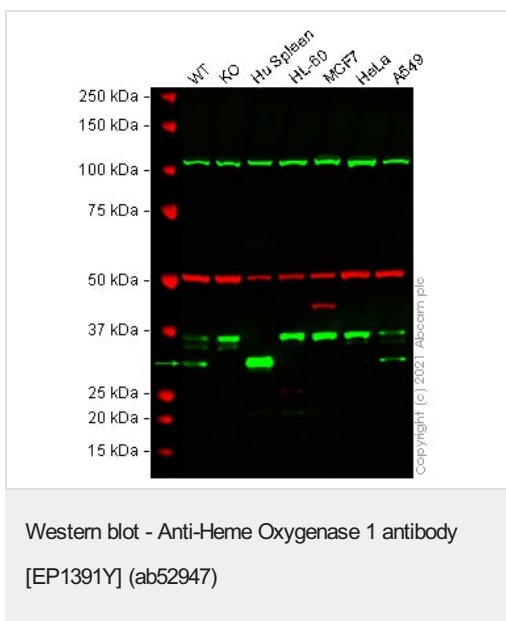
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (4)	1/2000. Detects a band of approximately 33 kDa (predicted molecular weight: 33 kDa).
IHC-P		Use a concentration of 0.1 - 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		1/30.

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 sequestered and destroyed.
Sequence similarities	Belongs to the heme oxygenase family.
Cellular localization	Microsome. Endoplasmic reticulum.

Images



All lanes : Anti-Heme Oxygenase 1 antibody [EP1391Y] (ab52947) at 1/2000 dilution

Lane 1 : Wild-type A549 cell lysate

Lane 2 : HMOX1 knockout A549 cell lysate

Lane 3 : Human Spleen tissue lysate

Lane 4 : HL-60 cell lysate

Lane 5 : MCF7 cell lysate

Lane 6 : HeLa cell lysate

Lane 7 : A549 cell lysate

Lysates/proteins at 20 µg per lane.

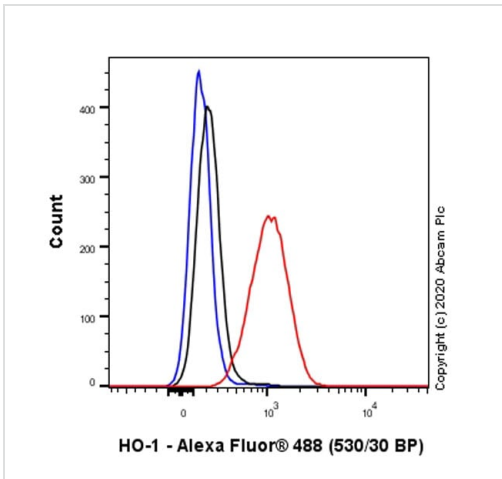
Performed under reducing conditions.

Predicted band size: 33 kDa

Observed band size: 33 kDa

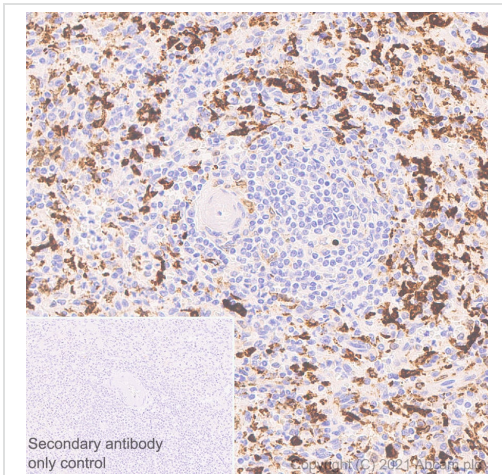
Lanes 1 - 7: Merged signal (red and green). Green - ab52947 observed at 33 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab52947 was shown to react with Heme Oxygenase 1 in wild-type A549 cells in Western blot with loss of signal observed in HMOX1 knockout cell line **ab269503** (knockout cell lysate **ab269665**). Wild-type A549 and HMOX1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab52947 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-Heme Oxygenase 1 antibody [EP1391Y] (ab52947)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (Mouse embryonic fibroblast) cells labelling Heme Oxygenase 1 with ab52947 at 1/50 dilution (1ug) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

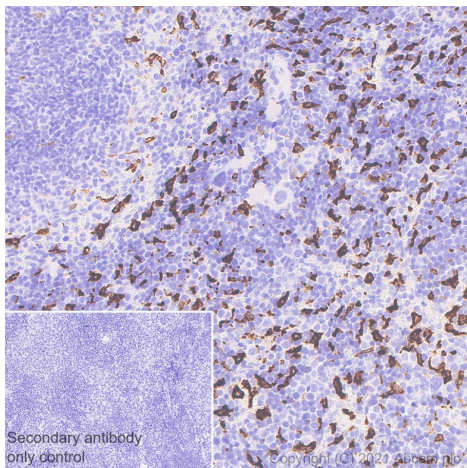


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Heme Oxygenase 1 antibody [EP1391Y] (ab52947)

Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling Heme Oxygenase 1 with ab52947 at 1/2000 (0.246 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond Polymer Refine Detection) was used. Cytoplasmic staining on human spleen. The section was incubated with ab52947 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

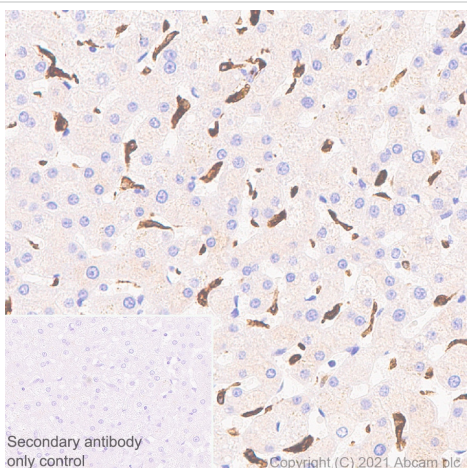


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Heme Oxygenase 1 antibody [EP1391Y] (ab52947)

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling Heme Oxygenase 1 with ab52947 at 1/2000 (0.246 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond Polymer Refine Detection) was used. Cytoplasmic staining on mouse spleen. The section was incubated with ab52947 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

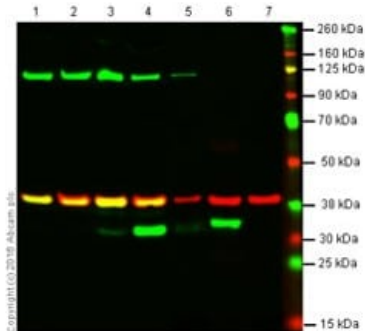


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Heme Oxygenase 1 antibody [EP1391Y] (ab52947)

Immunohistochemical analysis of paraffin-embedded Human liver tissue labeling Heme Oxygenase 1 with ab52947 at 1/2000 (0.246 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond Polymer Refine Detection) was used. Cytoplasmic staining on Kupffer cells in human liver. The section was incubated with ab52947 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Western blot - Anti-Heme Oxygenase 1 antibody [EP1391Y] (ab52947)

All lanes : Anti-Heme Oxygenase 1 antibody [EP1391Y] (ab52947) at 1/1000 dilution

Lane 1 : Hek293

Lane 2 : HL60

Lane 3 : HeLa

Lane 4 : A549

Lane 5 : Hu spleen

Lane 6 : Ms spleen

Lane 7 : Rt spleen

Lysates/proteins at 10 µg per lane.

Secondary

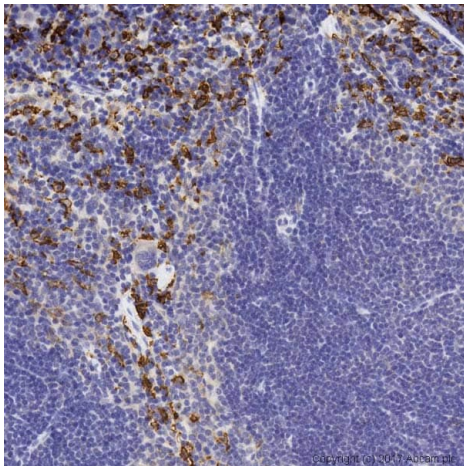
All lanes : IRDye® 800CW Goat anti Rabbit

Predicted band size: 33 kDa

Observed band size: 32 kDa

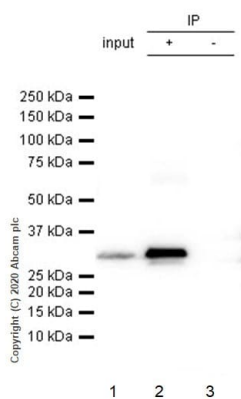
Hek293 & HL60 presumed negative or very low expression.

Loading control GAPDH at 38kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Heme Oxygenase 1 antibody [EP1391Y] (ab52947)

IHC image of ab52947 staining in mouse spleen formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol B. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab52947, 5µg/ml, for 15 mins at room temperature. A goat anti-rabbit biotinylated secondary antibody was used to detect the primary, and visualized using an HRP conjugated ABC system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



Immunoprecipitation - Anti-Heme Oxygenase 1 antibody [EP1391Y] (ab52947)

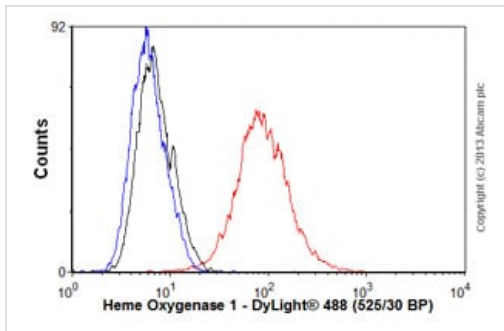
Heme Oxygenase 1 was immunoprecipitated from 0.35mg mouse spleen lysate with ab52947 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab52947 at 1/1000 dilution (1 µg/mL). VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used as the secondary antibody at 1/1000 dilution.

Lane 1: Mouse spleen tissue lysate 10 µg

Lane 2: Mouse spleen tissue lysate

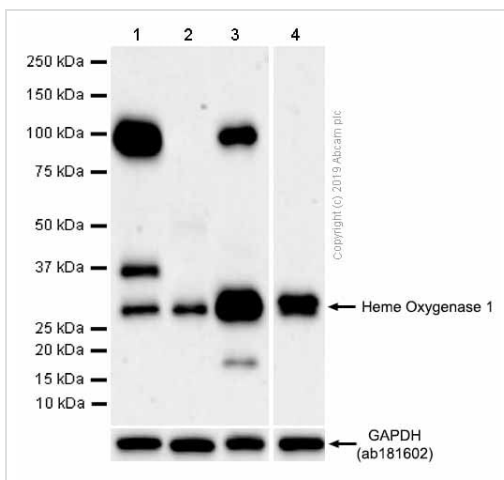
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab52947 in mouse spleen lysate.

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



Flow Cytometry (Intracellular) - Anti-Heme Oxygenase 1 antibody [EP1391Y] (ab52947)

Overlay histogram showing HEK-293 (human epithelial cell line from embryonic kidney) cells stained with ab52947 (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 followed by the antibody (ab52947, 1/1000 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) (0.1µg/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in HEK293 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Western blot - Anti-Heme Oxygenase 1 antibody [EP1391Y] (ab52947)

All lanes : Anti-Heme Oxygenase 1 antibody [EP1391Y] (ab52947) at 1/1000 dilution

Lane 1 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysates with 5% NFDm/TBST

Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates with 5% NFDm/TBST

Lane 3 : A549 (Human lung carcinoma epithelial cell) whole cell lysates with 5% NFDm/TBST

Lane 4 : Mouse spleen lysates with 5% NFDm/TBST

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Predicted band size: 33 kDa

Observed band size: 33 kDa

Exposure time: 120 seconds

We are unsure how to define the extra bands

Why choose a recombinant antibody?



- Research with confidence**
Consistent and reproducible results
- Long-term and scalable supply**
Recombinant technology
- Success from the first experiment**
Confirmed specificity
- Ethical standards compliant**
Animal-free production

Anti-Heme Oxygenase 1 antibody [EP1391Y]
(ab52947)

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