abcam

Product datasheet

Anti-Heme Oxygenase 1 antibody [EP1391Y] - BSA and Azide free ab219360



יישעבעדער RabMAb

9 References 画像数9

製品の概要

製品名 Anti-Heme Oxygenase 1 antibody [EP1391Y] - BSA and Azide free

製品の詳細 Rabbit monoclonal [EP1391Y] to Heme Oxygenase 1 - BSA and Azide free

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt (Intra), WB, IHC-P, IP

種交差性 交差種: Mouse. Human

交差が予測される動物種: Guinea pig

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

WB: Fetal liver lysate, human liver tissue, human spleen tissue, HL-60, MCF7, A549, NIH/3T3, ポジティブ・コントロール HEK-293, and HeLa cells. IHC-P: FFPE mouse spleen normal. Human spleen tissue. IP: Mouse

spleen tissue lysate. Flow Cyt (intra): NIH/3T3 cells.

特記事項 ab219360 is the carrier-free version of ab52947.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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 see here.

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**[®] **patents**.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C. Do Not Freeze.

バッファー pH: 7.20

Constituent: PBS

キャリア・フリー はい

精製度 Protein A purified

ポリ/モノ モノクローナル **クローン名** EP1391Y

アイソタイプ lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab219360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検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab199376</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 33 kDa (predicted molecular weight: 33 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.

ターゲット情報

機能 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

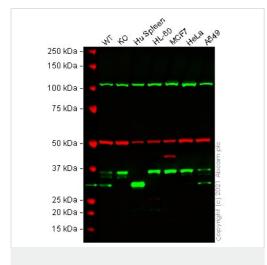
activity of heme oxygenase is highest in the spleen, where senescent erythrocytes are

sequestrated and destroyed.

配列類似性 Belongs to the heme oxygenase family.

細胞内局在 Microsome. Endoplasmic reticulum.

画像



Western blot - Anti-Heme Oxygenase 1 antibody [EP1391Y] - BSA and Azide free (ab219360)

All lanes: Anti-Heme Oxygenase 1 antibody [EP1391Y]

(ab52947) at 1/1000 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.

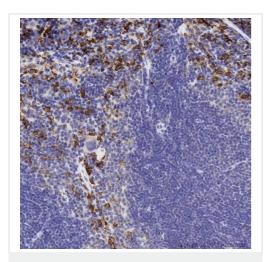
Performed under reducing conditions.

Predicted band size: 33 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab52947</u>).

Lanes 1 - 7: Merged signal (red and green). Green - <u>ab52947</u> observed at 33 kDa. Red - loading control <u>ab7291</u> (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.

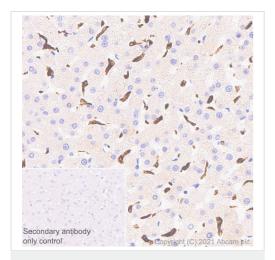


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Heme Oxygenase 1 antibody [EP1391Y] - BSA and Azide free (ab219360)

IHC image of <u>ab52947</u> staining in mouse spleen formalin fixed paraffin embedded tissue section, performed on a Leica

Bond™ system using the standard protocol B. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with <u>ab52947</u>, 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52947).



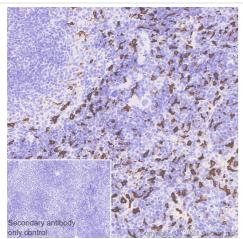
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Heme Oxygenase 1 antibody [EP1391Y] - BSA and Azide free (ab219360)

This data was developed using <u>ab52947</u>, the same antibody clone in a different buffer formulation.

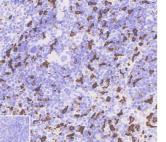
Immunohistochemical analysis of paraffin-embedded Human liver tissue labeling Heme Oxygenase 1 with ab52947 at 1/2000 (0.246 ug/ml) followed by a LeicaDS9800 (Bond Polymer Refine Detection) was used at Ready to use dilution. Cytoplasmic staining on Kupffer cells in human liver. The section was incubated 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 LeicaDS9800 (Bond™ Polymer Refine Detection) was used at Ready to use dilution.

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Heme Oxygenase 1 antibody [EP1391Y] - BSA and Azide free (ab219360)



Secondary antibody only control: Secondary antibody is LeicaDS9800 (Bond™ Polymer Refine Detection) was used at Ready to use dilution.

Leica Biosystems BOND RX instrument Counterstained with

This data was developed using <u>ab52947</u>, the same antibody clone

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling Heme Oxygenase 1 with ab52947 at 1/2000 (0.246

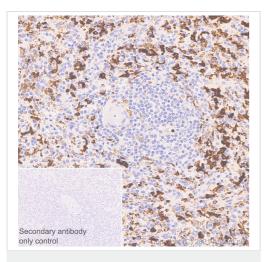
Detection) was used at Ready to use dilution. Cytoplasmic staining on mouse spleen. The section was incubated with ab52947 for 30 mins at room temperature. The immunostaining was performed on a

ug/ml) followed by a LeicaDS9800 (Bond Polymer Refine

in a different buffer formulation.

Hematoxylin.

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



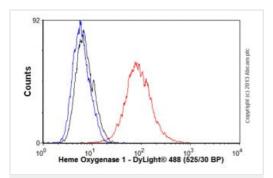
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Heme Oxygenase 1 antibody [EP1391Y] - BSA and Azide free (ab219360)

This data was developed using ab52947, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling Heme Oxygenase 1 with ab52947 at 1/2000 (0.246 ug/ml) followed by a LeicaDS9800 (Bond Polymer Refine Detection) was used at Ready to use dilution. 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 LeicaDS9800 (Bond Polymer Refine Detection) was used at Ready to use dilution.

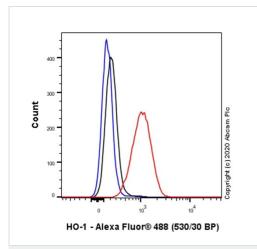
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Flow Cytometry (Intracellular) - Anti-Heme

Oxygenase 1 antibody [EP1391Y] - BSA and Azide free (ab219360)

Overlay histogram showing HEK293 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 lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (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. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52947).

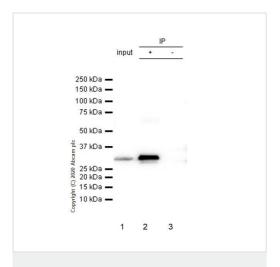


Flow Cytometry (Intracellular) - Anti-Heme

Oxygenase 1 antibody [EP1391Y] - BSA and Azide free (ab219360)

This data was developed using <u>ab52947</u>, the same antibody clone in a different buffer formulation.

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.



Immunoprecipitation - Anti-Heme Oxygenase 1 antibody [EP1391Y] - BSA and Azide free (ab219360) Heme Oxygenase 1 was immunoprecipitated from 0.35mg mouse spleen lysate with <u>ab52947</u> at 1/30 dilution (2 μ g in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab52947</u> at 1/1000 dilution (1 μ g/mL). VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) 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 lgG (<u>ab172730</u>) instead of <u>ab52947</u> in mouse spleen lysate.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52947).



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