abcam

Product datasheet

Anti-Galactosidase alpha antibody [EP5828(2)] ab168341



ייבעדיו RabMAb

★★★★★ 2 Abreviews 8 References 画像数 12

製品の概要

製品名 Anti-Galactosidase alpha antibody [EP5828(2)]

製品の詳細 Rabbit monoclonal [EP5828(2)] to Galactosidase alpha

由来種 Rabbit

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

種交差性 交差種: Human

免疫原 Synthetic peptide within Human Galactosidase alpha aa 100-200. The exact sequence is

proprietary.

Database link: P06280

ポジティブ・コントロール WB: MCF-7, 293T, A431, HAP1 and HeLa whole cell lysate (ab150035). IHC-P: Human urinary

bladder carcinoma, kidney and uterus tissue. ICC/IF: HeLa cells IP: MCF-7 cell lysates. Flow Cyt

(intra): HeLa cells.

特記事項 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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

バッファー Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

精製度 Protein A purified

ポリ/モノ モノクローナル **ウローン名** EP5828(2)

ΙgG

アプリケーション

アイソタイプ

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ab168341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)		1/90.
IP		1/10 - 1/100.
WB	*** <u>*</u>	1/1000 - 1/10000. Predicted molecular weight: 49 kDa.
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
ICC/IF		1/50 - 1/500.

ターゲット情報

関連疾患

Defects in GLA are the cause of Fabry disease (FD) [MIM:301500]. FD is a rare X-linked sphingolipidosis disease where glycolipid accumulates in many tissues. The disease consists of an inborn error of glycosphingolipid catabolism. FD patients show systemic accumulation of globotriaoslyceramide (Gb3) and related glycosphingolipids in the plasma and cellular lysosomes throughout the body. Clinical recognition in males results from characteristic skin lesions (angiokeratomas) over the lower trunk. Patients may show ocular deposits, febrile episodes, and burning pain in the extremities. Death results from renal failure, cardiac or cerebral complications of hypertension or other vascular disease. Heterozygous females may exhibit the disorder in an attenuated form, they are more likely to show corneal opacities.

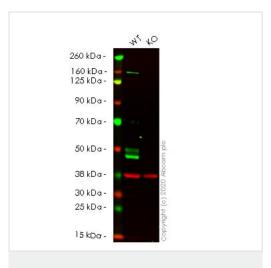
配列類似性

Belongs to the glycosyl hydrolase 27 family.

細胞内局在

Lysosome.

画像



Western blot - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)

All lanes : Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2: GLA knockout HeLa cell lysate

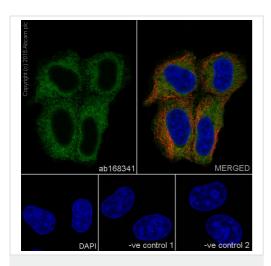
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 49 kDa Observed band size: 49 kDa

Lanes 1-2: Merged signal (red and green). Green - ab168341 observed at 49 kDa. Red - loading control <u>ab8245</u> observed at 37 kDa.

ab168341 Anti-Galactosidase alpha antibody [EP5828(2)] was shown to specifically react with Galactosidase alpha in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265563 (knockout cell lysate ab257449) was used. Wild-type and Galactosidase alpha knockout samples were subjected to SDS-PAGE. ab168341 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 and 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.



Immunocytochemistry/ Immunofluorescence - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)

260 kDa 160 kDa 125 kDa 70 kDa 38 kDa 30 kDa 25 kDa 15 kDa -

Western blot - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling Galactosidase with purified ab168341 at 1/500. Cells were fixed with 100% methanol and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor[®] 488-conjugated goat anti-rabbit lgG (1/1000) 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 lgG (1/1000) were also used.

Control 1: primary antibody (1/500) and secondary antibody, **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: $\underline{ab7291}$ (1/1000) and secondary antibody, $\underline{ab150077}$, an Alexa Fluor[®] 488-conjugated goat anti-rabbit lgG (1/1000).

All lanes : Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: GLA knockout HAP1 whole cell lysate

Lane 3: Hela whole cell lysate

Lane 4: MCF-7 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 49 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab168341 observed at 49 kDa. Red - loading control, <u>ab130007</u>, observed at 125 kDa.

ab168341 was shown to recognize GLA (Alpha-galactosidase A) in wild-type HAP1 cells as signal was lost at the expected MW in GLA knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and GLA knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% milk. Ab168341 and ab130007 (Mouse anti-

Vinculin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.

250 kDa — 150 kDa — 100 kDa — 75 kDa — 50 kDa — 25 kDa — 25 kDa — 25 kDa — 15 kDa — 15 kDa — 10 kDa —

Western blot - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)

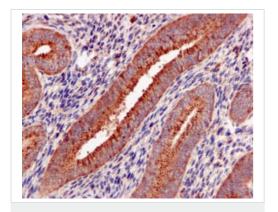
Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341) at 20 µg (purified) + MCF-7 whole cell lysate at 20 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 49 kDa **Observed band size:** 46 kDa

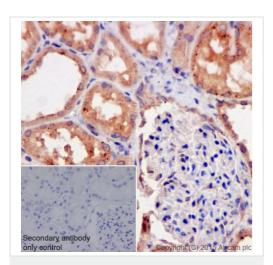
Blocking and dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)

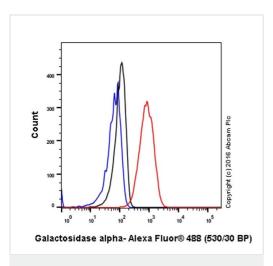
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human uterus tissue labelling Galactosidase alpha with unpurified ab168341 at a dilution of 1/50.

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



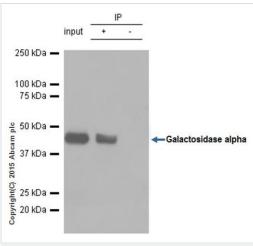
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue labelling Galactosidase alpha with purified ab168341 at 1/50. 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.

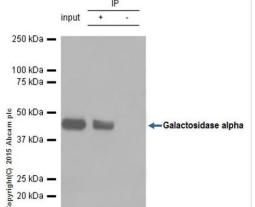


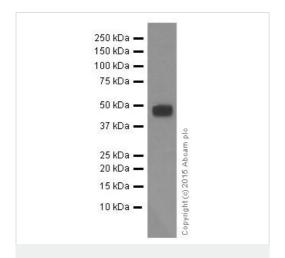
Flow Cytometry (Intracellular) - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)

Intracellular Flow Cytometry analysis ofHeLa cells labelling Galactosidase alpha with purified ab168341 at a dilution of 1/90 (red). Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. An Alexa Flour[®] 488-conjugated goat anti-rabbit lgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal lgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Immunoprecipitation - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)





Western blot - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)

ab168341 (purified) at 1/60 immunoprecipitating Galactosidase alpha in MCF-7 whole cell lysate.

Lane 1 (input): MCF-7 whole cell lysate (10µg)

Lane 2 (+): ab168341 + MCF-7 whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab168341 in MCF-7 whole cell lysate.

For western blotting, a HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

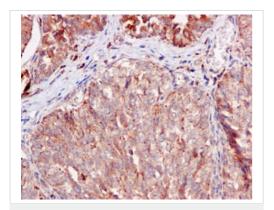
Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341) at 1/5000 dilution (purified) + HEK293 whole cell lysate at 10 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 49 kDa Observed band size: 46 kDa

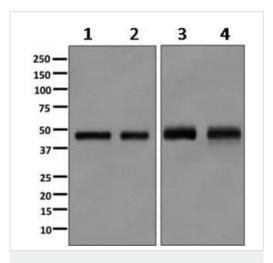
Blocking and dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human urinary bladder carcinoma tissue labelling Galactosidase alpha with unpurified ab168341 at a dilution of 1/50.

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



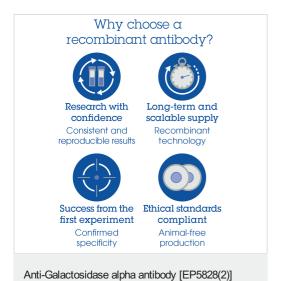
Western blot - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)

All lanes : Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341) at 1/1000 dilution (unpurified)

Lane 1: MCF-7 cell lysates
Lane 2: 293T cell lysates
Lane 3: A431 cell lysates
Lane 4: HeLa cell lysates

Lysates/proteins at 10 µg per lane.

Predicted band size: 49 kDa



(ab168341)

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