abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

2 Abreviews 8 References 12 Images

Overview

Product name	Anti-Galactosidase alpha antibody [EP5828(2)]
Description	Rabbit monoclonal [EP5828(2)] to Galactosidase alpha
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), IP, WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human Galactosidase alpha aa 100-200. The exact sequence is proprietary. Database link: **P06280**
Positive control	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.
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 **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.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide

	Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP5828(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab168341 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)		1/90.
IP		1/10 - 1/100.
WB	★★★★★ (1)	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.

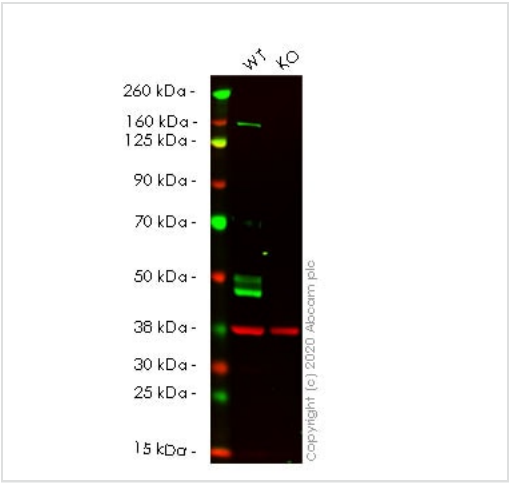
Target

Involvement in disease 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.

Sequence similarities Belongs to the glycosyl hydrolase 27 family.

Cellular localization Lysosome.

Images



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

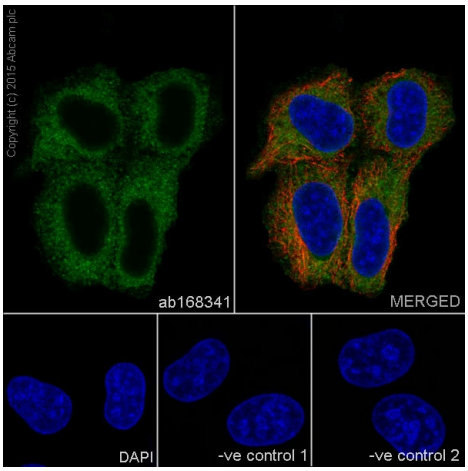
Lysates/proteins at 20 µg per lane.

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 **ab8245** 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.

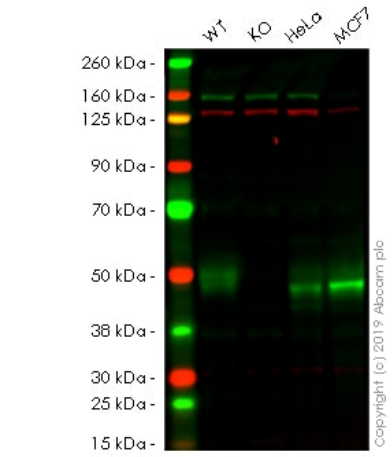


Immunocytochemistry/ Immunofluorescence - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)

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



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 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

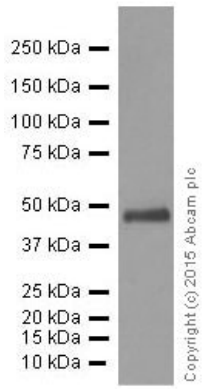
Lysates/proteins at 20 µg per lane.

Predicted band size: 49 kDa

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



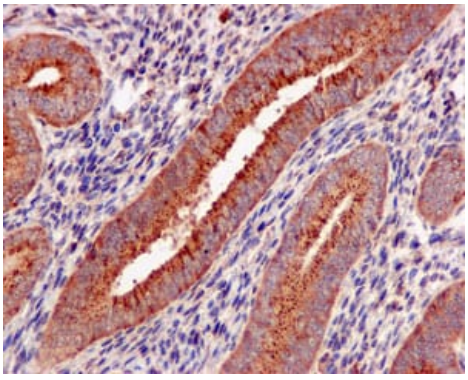
Western blot - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)

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

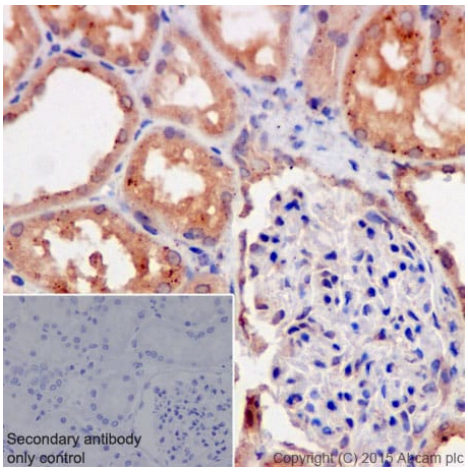
Blocking and dilution buffer: 5% NFDM/TBST.



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

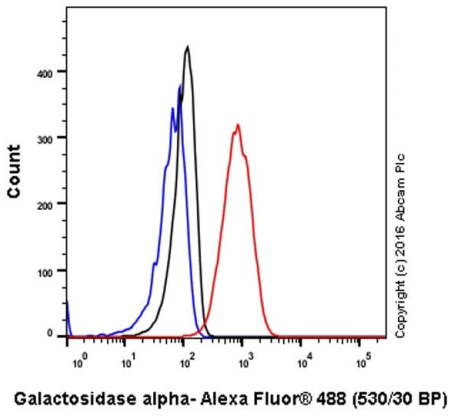
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human uterus tissue labelling Galactosidase alpha with unpurified ab168341 at a dilution of 1/50.

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



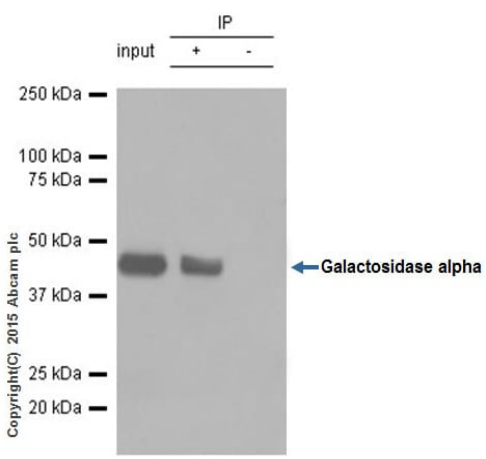
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Galactosidase alpha antibody [EP5828(2)] (ab168341)

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 IgG (1/2000) 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-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)

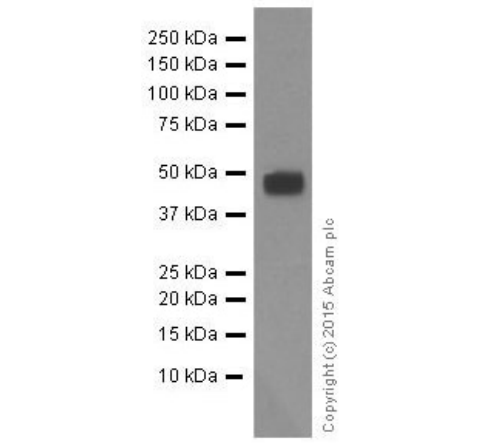
Lane 2 (+): ab168341 + MCF-7 whole cell lysate.

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

For western blotting, a HRP-conjugated anti-rabbit IgG, 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.



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

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

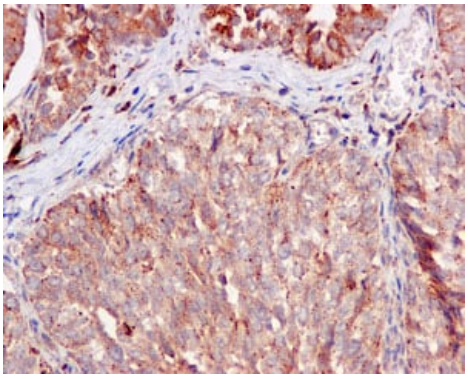
Secondary

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

Predicted band size: 49 kDa

Observed band size: 46 kDa

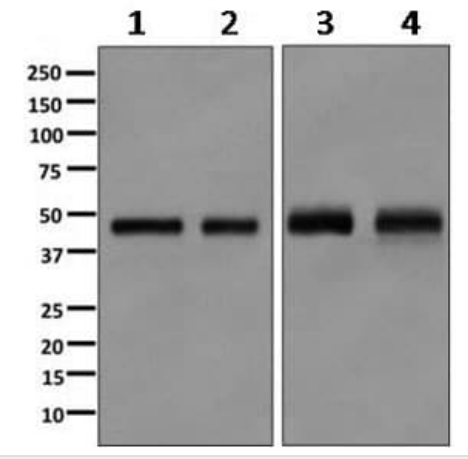
Blocking and dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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

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

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