abcam

Product datasheet

Anti-Glypican 3 antibody [SP86] ab95363

KO VALIDATED Recombinant RabMAb

6 References 10 Images

Overview

Product name	Anti-Glypican 3 antibody [SP86]
Description	Rabbit monoclonal [SP86] to Glypican 3
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, Flow Cyt, WB, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human Glypican 3 aa 500 to the C-terminus (C terminal). The exact sequence is proprietary. Database link: **P51654**
Positive control	Human liver cancer tissueThis antibody gave a positive result when used in the following formaldehyde fixed cell lines: HepG2. ICC/IF: HepG2 Flow Cyt: HepG2, Hap1 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**. **This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.**

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.1% Sodium azide Constituents: 1% BSA, PBS
Purity	Protein A purified

Clonality	Monoclonal
Clone number	SP86
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab95363 in the following tested applications.

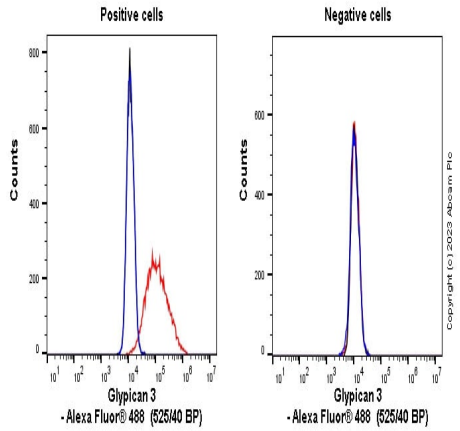
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/200.
Flow Cyt		1/80. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Predicted molecular weight: 66 kDa.
IHC-P		1/100. Perform heat mediated antigen retrieval (boil tissue section in 10mM citrate buffer, pH 6.0 for 10 min followed by cooling at RT for 20 min).

Target

Function	Cell surface proteoglycan that bears heparan sulfate. Inhibits the dipeptidyl peptidase activity of DPP4. May be involved in the suppression/modulation of growth in the predominantly mesodermal tissues and organs. May play a role in the modulation of IGF2 interactions with its receptor and thereby modulate its function. May regulate growth and tumor predisposition.
Tissue specificity	Highly expressed in lung, liver and kidney.
Involvement in disease	Defects in GPC3 are the cause of Simpson-Golabi-Behmel syndrome type 1 (SGBS1) [MIM:312870]; also known as Simpson dysmorphia syndrome (SDYS). SGBS is a condition characterized by pre- and postnatal overgrowth (gigantism) with visceral and skeletal anomalies.
Sequence similarities	Belongs to the glypican family.
Cellular localization	Cell membrane and Secreted > extracellular space.

Images



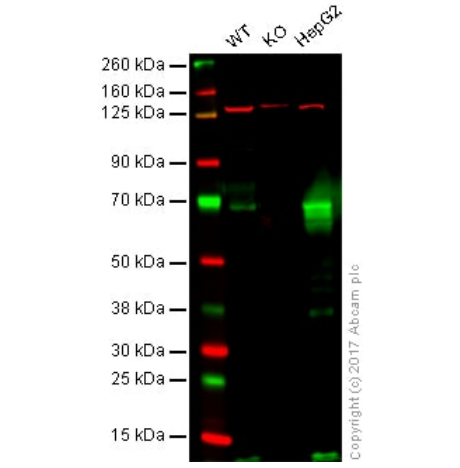
Flow Cytometry - Anti-Glypican 3 antibody [SP86] (ab95363)

Flow cytometry overlay histogram showing left wild-type Hap1 positive cells and right negative GPC3 knockout Hap1 stained with ab95363 (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab95363) (1x 10^6 in 100µl at 5.0 µg/ml (1/396)) for 30min on ice.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min on ice

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (black line) was used at the same concentration and conditions as the primary antibody. Unlabelled sample was also used as a control (blue line).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

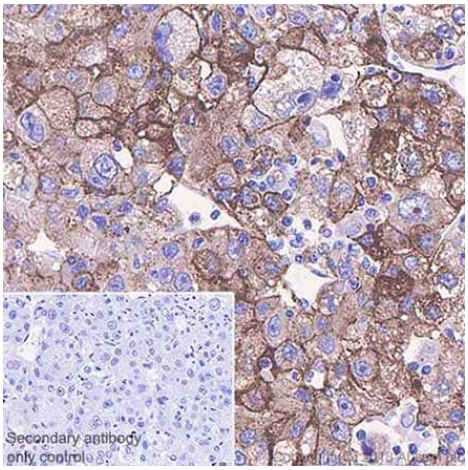


Western blot - Anti-Glypican 3 antibody [SP86] (ab95363)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: GPC3 knockout HAP1 whole cell lysate (20 µg)
Lane 3: HepG2 whole cell lysate (20 µg)

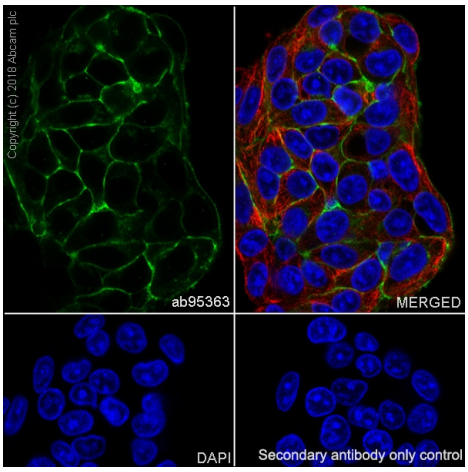
Lanes 1 - 3: Merged signal (red and green). Green - ab95363 observed at 70 kDa. Red - loading control, **ab130007**, observed at 125kDa.

ab95363 was shown to specifically react with Glypican 3 in wild-type HAP1 cells as signal was lost in GPC3 knockout cells. Wild-type and GPC3 knockout samples were subjected to SDS-PAGE. ab95363 and **ab130007** (Mouse anti-vinculin loading control) were incubated overnight at 4°C at 1/1000 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 1/10000 dilution for 1 hour at room temperature before imaging.



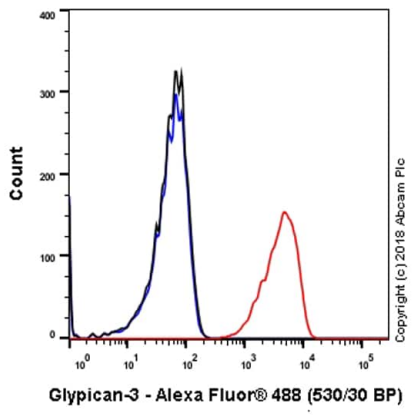
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Glypican 3 antibody [SP86] (ab95363)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human hepatocellular carcinoma tissue sections labeling Glypican 3 with ab95363 at 1:100 dilution (4.66 ? g/ml). Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 10mins. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Hematoxylin was used as a counterstain. Positive staining on human hepatocellular carcinoma, performed on a Leica Biosystems BOND™ RX instrument.
The section was incubated with ab95363 for 30 mins at room temperature.



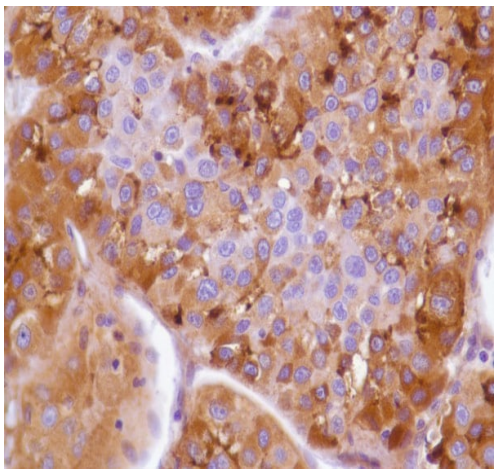
Immunocytochemistry/ Immunofluorescence - Anti-Glypican 3 antibody [SP86] (ab95363)

Immunocytochemistry/ Immunofluorescence analysis of HepG2 (human hepatocellular carcinoma epithelial cell) cells labeling Glypican 3 with purified ab95363 at 1/200 (2.3 µg/ml). Cells were fixed in 100% Methanol and permeabilized with None. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



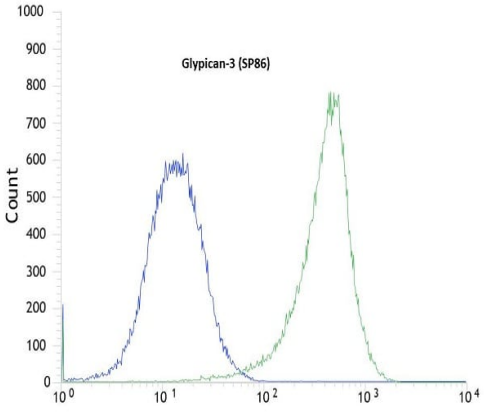
Flow Cytometry - Anti-Glypican 3 antibody [SP86] (ab95363)

Flow cytometry analysis of HepG2 (human hepatocellular carcinoma) labeling Glypican 3 with purified ab95363 at 1/80 dilution (5.825 µg/ml) (red). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as a secondary antibody. Isotype control - Rabbit monoclonal IgG (**ab172730**) (Black). Unlableled control -Unlabelled cells (blue).



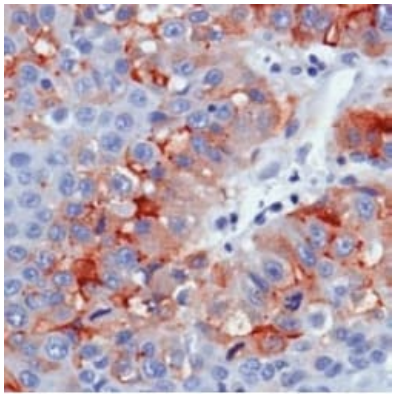
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Glypican 3 antibody [SP86] (ab95363)

Immunohistochemical staining of human liver hepatocellular carcinoma with ab95363.



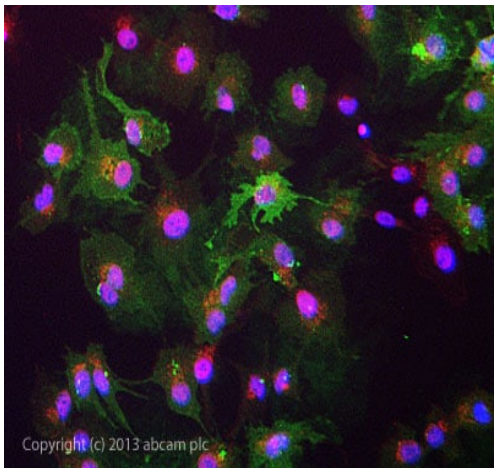
Flow Cytometry - Anti-Glypican 3 antibody [SP86] (ab95363)

Flow cytometric analysis of rabbit anti-Glypican 3 (SP86) antibody **ab98363** (1/100) in HEPG2 cellls (green) compared to negative control of rabbit IgG (blue).



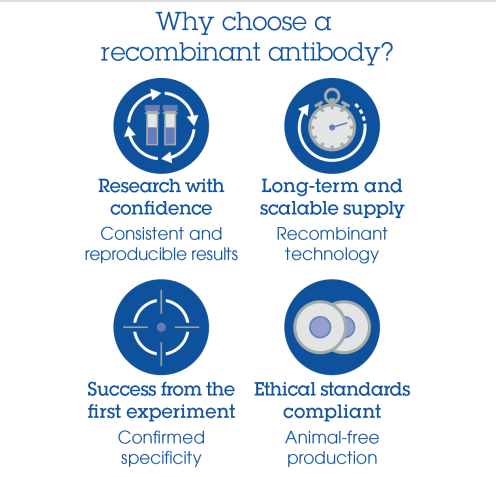
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Glypican 3 antibody [SP86] (ab95363)

ab95363, at 1/100 dilution, staining Glypican 3 in formalin-fixed, paraffin-embedded Human liver cancer tissue by Immunohistochemistry.



Immunocytochemistry/ Immunofluorescence - Anti-Glypican 3 antibody [SP86] (ab95363)

ICC/IF image of ab95363 stained HepG2 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab95363 at 5µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit (**ab96899**) IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Anti-Glypican 3 antibody [SP86] (ab95363)

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