abcam

ab95363

Anti-Glypican 3 antibody [SP86]

Anti-Glypican 3 antibody [SP86] (ab95363) is a rabbit monoclonal antibody detecting Glypican 3 in **Western Blot, Flow Cytometry, IHC-P, ICC/IF**. Suitable for **Human**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Trusted since 2010

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	SP86
Purification technique	Affinity purification Protein A
Concentration	0.466 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt

Tested

Species	Human
Dilution info	1/80
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

WB

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/100
Notes	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).

ICC/IF

Tested

Species	Human
Dilution info	1/200
Notes	-

Target data

See full target information GPC3

Function	Cell surface proteoglycan (PubMed:14610063). Negatively regulates the hedgehog signaling pathway when attached via the GPI-anchor to the cell surface by competing with the hedgehog receptor PTC1 for binding to hedgehog proteins (By similarity). Binding to the hedgehog protein SHH triggers internalization of the complex by endocytosis and its subsequent lysosomal degradation (By similarity).

Positively regulates the canonical Wnt signaling pathway by binding to the Wnt receptor Frizzled and stimulating the binding of the Frizzled receptor to Wnt ligands (PubMed:16227623, PubMed:24496449). Positively regulates the non-canonical Wnt signaling pathway (By similarity). Binds to CD81 which decreases the availability of free CD81 for binding to the transcriptional repressor HHEX, resulting in nuclear translocation of HHEX and transcriptional repression (By similarity). Inhibits the dipeptidyl peptidase activity of DPP4 (PubMed:17549790). Plays a role in limb patterning and skeletal development by controlling the cellular response to BMP4 (By similarity). Modulates the effects of growth factors BMP2, BMP7 and FGF7 on renal branching morphogenesis (By similarity). Required for coronary vascular development (By similarity). Plays a role in regulating cell movements during gastrulation (By similarity).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-Glypican 3 antibody [SP86] (ab95363) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Flow Cyt), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human samples.

What is the molecular weight of Glypican 3?
Anti-Glypican 3 [SP86] (ab95363) specifically detects a band for Glypican 3 (UniProt: P51654) at a molecular weight of 66kDa.

Collaboration
Anti-Glypican 3 [SP86] (ab95363) is a clone from the portfolio of Spring Bioscience (Roche) SP clones which have been optimised for immunohistochemistry (IHC).

Specificity confirmed
The specificity of Anti-Glypican 3 antibody [SP86] (ab95363) has been confirmed by Western blot testing in GPC3 Knockout HAP1 cells.

Other related products
We have a range of other formats of antibody clone [SP86] also available for your convenience: ab95363, Carrier free

- ab238804, Alexa Fluor® 647 - ab282189, PE - ab303587, Alexa Fluor® 488 - ab314880

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Glypican 3 also known as GPC3 is a cell surface heparan sulfate proteoglycan with a core protein mass of approximately 70 kDa. This target is anchored to the external cell membrane through a glycosylphosphatidylinositol (GPI) anchor. Glypican 3 is expressed mainly in tissues such as the liver and kidneys and plays a role in modulating cell growth and development. In research studies it is often studied in various species including in the mouse and cynomolgus monkey models where it's referred to as mouse glypican 3 and cynomolgus glypican 3 respectively.
Biological function summary	Glypican 3 is involved in the modulation of signaling pathways that control cell proliferation and differentiation. It does not function as an isolated element; it has a role as part of a complex network that interacts with growth factors morphogens and other proteoglycans. Glypican 3 assists in the regulation of important biological events by influencing the accessibility and activity of signaling molecules on the cell surface. Its heparan sulfate chains interact with multiple proteins modifying their function and availability.
Pathways	Glypican 3 plays a significant part in the Wnt and Hedgehog signaling pathways. In the Wnt pathway it regulates the distribution and gradient formation of Wnt proteins which are essential for proper embryonic development. In the Hedgehog pathway glypican 3 modulates the distribution and activity of Hedgehog proteins important for tissue patterning and organogenesis. Other proteins closely related to glypican 3 in these pathways include those from the frizzled family in the Wnt pathway and Patched proteins in the Hedgehog pathway.
Associated diseases and disorders	Glypican 3 has a strong connection to hepatocellular carcinoma (HCC) and Simpson-Golabi-Behmel syndrome (SGBS). In HCC increased expression of glypican 3 is often observed which associates it with tumor growth and proliferation. It functions alongside other oncogenic factors like alpha-fetoprotein (AFP) which is another marker for liver cancer. In SGBS mutations in the GPC3 gene result in defects in cellular signaling pathways leading to developmental abnormalities. This syndrome highlights the impact of glypican 3 on growth regulation and its interactions with insulin-like growth factors (IGFs).

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

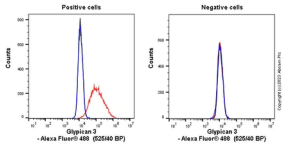
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

9 product images



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

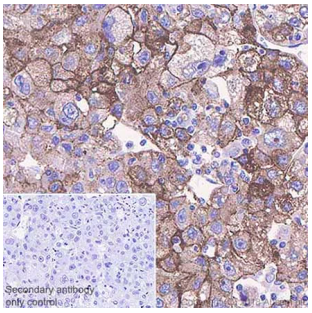
Glypican 3 Flow Cytometry staining using rabbit Anti-Glypican 3 antibody

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 106 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).

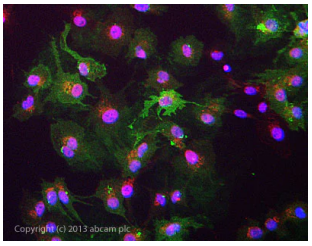
Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



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

Glypican 3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Glypican 3 antibody

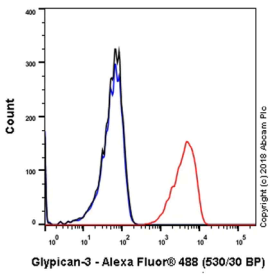
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human hepatocellular carcinoma tissue sections labeling Glypican 3 with ab95363 at 1:100 dilution (4.66 µg/ml). Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 10mins. 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)

Glypican 3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Glypican 3 antibody

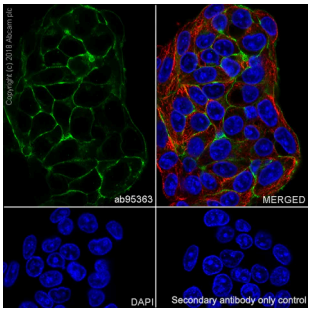
ICC/IF image of ab95363 stained HepG2 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the 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.



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

Glypican 3 Flow Cytometry staining using rabbit Anti-Glypican 3 antibody

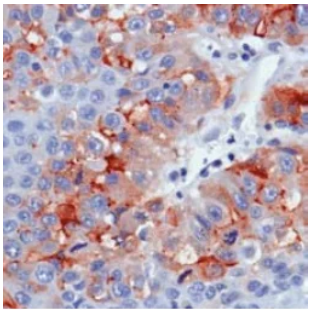
Flow cytometry analysis of HepG2 (human hepatocellular carcinoma) labeling Glypican 3 with purified ab95363 at 1/80 dilution (5.825 µg/ml) (red). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as a secondary antibody. Isotype control - Rabbit monoclonal IgG (ab172730) (Black).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



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

Glypican 3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Glypican 3 antibody

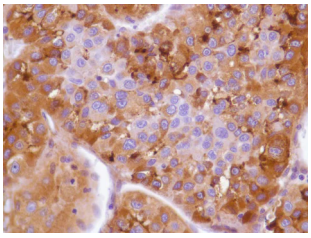
Immunocytochemistry/ Immunofluorescence analysis of HepG2 (human hepatocellular carcinoma epithelial cell) cells labeling Glypican 3 with purified ab95363 at 1/200 (2.3 µg/ml). Cells were fixed in 100% Methanol and permeabilized with None. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [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.



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

Glypican 3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Glypican 3 antibody

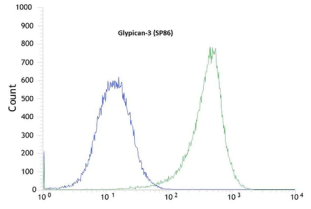
ab95363, at 1/100 dilution, staining Glypican 3 in formalin-fixed, paraffin-embedded Human liver cancer tissue by Immunohistochemistry.



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

Glypican 3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Glypican 3 antibody

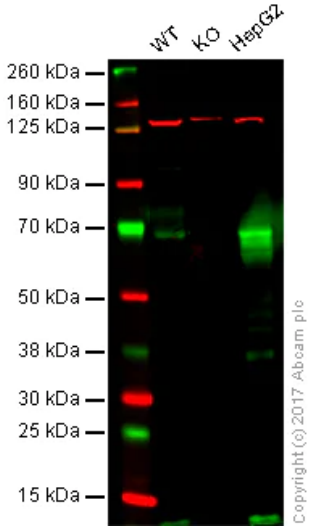
Immunohistochemical staining of human liver hepatocellular carcinoma with ab95363.



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

Glypican 3 Flow Cytometry staining using rabbit Anti-Glypican 3 antibody

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).



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

Glypican 3 Western blot staining using rabbit Anti-Glypican 3 antibody

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.

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

Predicted band size: 66 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.