Human Glypican 3 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF2119

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Glypican 3 in direct ELISAs and Western blots. In direct ELISAs, less than 10% cross-reactivity with recombinant mouse Glypican 3 is observed and less than 5% cross-reactivity with recombinant human (rh) Glypican 2, rhGlypican 5, and rhGlypican 6 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Glypican 3 Gln25-Val558 Accession # P51654
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

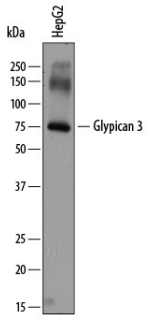
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	2 µg/mL	See Below
Flow Cytometry	2.5 µg/10^6 cells	HepG2 human hepatocellular carcinoma cell line
Immunocytochemistry	1-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

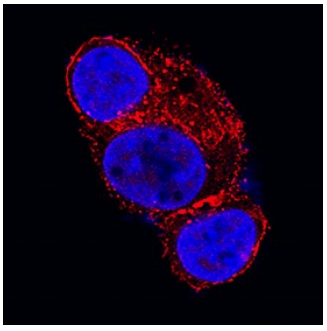
DATA

Western Blot



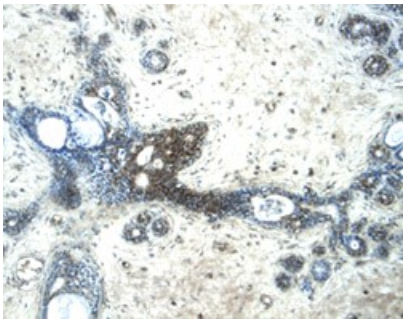
Detection of Human Glypican 3 by Western Blot. Western blot shows lysates of HepG2 human hepatocellular carcinoma cell line. PVDF membrane was probed with 2 µg/mL of Sheep Anti-Human Glypican 3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2119) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Glypican 3 at approximately 75 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



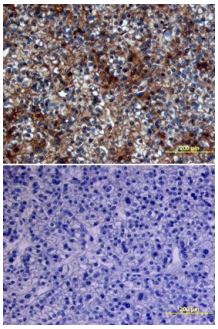
Glypican 3 in HepG2 Human Cell Line. Glypican 3 was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Sheep Anti-Human Glypican 3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2119) at 1.7 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm and cell membranes. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



Glypican 3 in Human Breast. Glypican 3 was detected in immersion fixed paraffin-embedded sections of human breast using 5 µg/mL Sheep Anti-Human Glypican 3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2119) overnight at 4 °C. Tissue was stained with the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Immunohistochemistry



Glypican 3 in Human Liver Cancer Tissue. Glypican 3 was detected in immersion fixed paraffin-embedded sections of human liver cancer tissue using Sheep Anti-Human Glypican 3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2119) at 10 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

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Catalog Number: AF2119

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Glypicans (GPC) are a family of heparan sulfate proteoglycans that are attached to the cell surface by a glycosylphosphatidylinositol (GPI) anchor. Six members of this family have been identified in mammals (GPC1-GPC6). All glypican core proteins contain an N-terminal signal peptide, a large globular cysteine-rich domain (CRD) with 14 invariant cysteine residues, a stalk-like region containing the heparan sulfate attachment sites, and a C-terminal GPI attachment site. While glypican proteins do not share strong amino acid sequence identity (they range from 17-63%), the conserved cysteine residues in their CRDs suggests similarity in their three-dimensional structure (1, 2).

Mutations in GPC3 cause a rare disorder in humans, Simpson-Golabi-Behmel Syndrome, which is characterized by pre and postnatal overgrowth of multiple tissues and organs and an increased risk for developing embryonic tumors (3). These features are also present in the mouse knock-out of GPC3 indicating that GPC3 regulates cell survival and inhibits cell proliferation during development (4). Glypican 3 has been implicated in regulating many different signaling pathways including: IGF, FGF, BMP, and Wnt. An endoproteolytic processing of GPC3 by proprotein convertases is required for the modulation of Wnt signaling (5). Direct interaction with FGF-basic has been observed and is mediated by the heparan sulfate chains (6).

References:

1. Filmus, J. and S.B. Selleck (2001) J. Clinical Invest. **108**:497.
2. De Cat, B and G. David (2001) Seminars in Cell & Dev. Biol. **12**:117.
3. Pilia, G. *et al.* (1996) Nat. Genet. **12**: 241.
4. Cano-Gauci, D.F. *et al.* (1999) J. Cell Biol. **146**: 255.
5. De Cat, B. *et al.* (2003) J. Cell Biol. **163**:625.
6. Song, H.H. *et al.* (1997) J. Biol. Chem. **272**:7574.

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