Product Datasheet

Anti-Glypican 1 (GPC1) [IPI-GPC1.44]

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Overview

Antigen	Glypican 1 (GPC1)
Immunogen	Purified recombinant fragment of Human Glypican 1 (GPC1), AA: 23-497.
Host/isotype	Rabbit/IgG1
Clonality	Recombinant monoclonal
Clone name	IPI-GPC1.44
RRID	AB_3101859
IPI ID	TAB0010753-013-002
Specificity	GPC1; Does not recognize other GPCs
Species reactivity	Human
Amount	100 µg
Concentration	1 mg/mL
Purification	Expressed in ExpiCHO cells and affinity purified using Protein A
Storage buffer	PBS, pH 7.4 with 0.02% ProClin 300
Shipping	Shipped on blue ice at +4C
Storage	Store at +4C for up to 3 months. For long-term storage, aliquot and store at -20C. Avoid multiple freeze/thaw cycles.

IPI Tested Applications‡

Application	Tested concentration	Result	Reference
Flow	5 µg/mL	Positive	https://doi.org/10.57733/addgene.r3dpk8
IF	2 µg/mL	Positive	https://doi.org/10.57733/addgene.03a3kp
WB	1 µg/mL	Negative	Unpublished

‡ Not suitable for WB application.

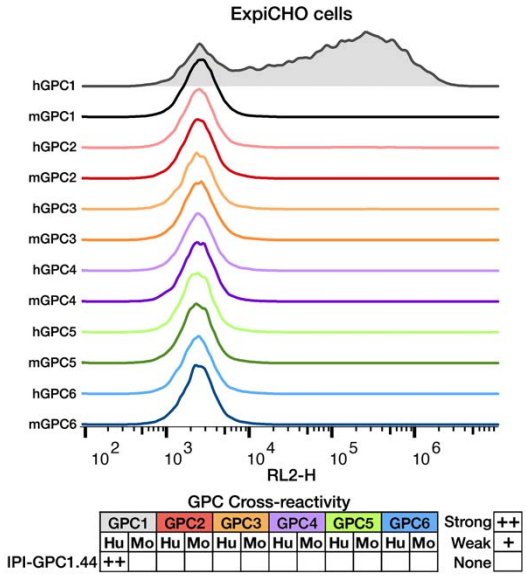
Community Data*

Application	Lab	Reference
IF	Adrian Salic, Ph.D., Harvard Medical School	https://doi.org/10.57733/addgene.4203q1

* Supporting Data is generated by external partner labs, in the process of evaluating IPI antibody panels.

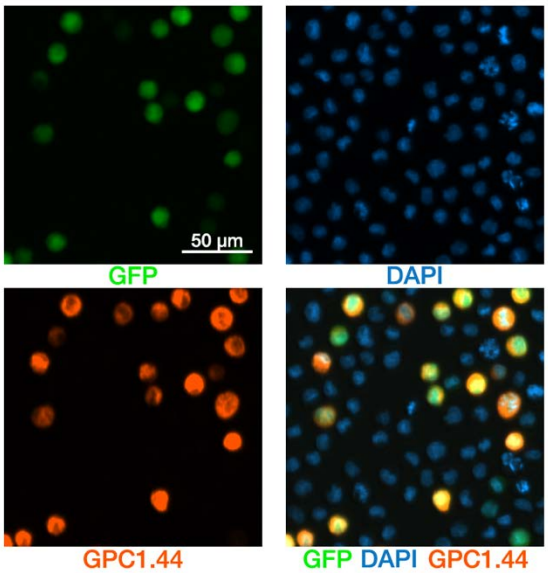
Applications

Flow cytometry



Histogram overlays from FACS analysis on ExpiCHO cells transfected with human and mouse glypicans (GPC1-GPC6). Cells expressing GPCs were labeled with Anti-Glypican 1 (GPC1) [IPI-GPC1.44] (Addgene #222512) and Alexa Fluor 647 F(ab')2 Fragment Goat Anti-Rabbit IgG Fc (Jackson ImmunoResearch, 111-606-046). Labeled cells were analyzed with an Intellicyt iQue Screener Plus flow cytometer. Anti-Glypican 1 (GPC1) [IPI-GPC1.44] is specific for human GPC1 (black line with gray fill). Histograms were normalized to mode and displayed as an overlay with half offset using FlowJo™ v10.10. Bottom table summarizes GPC cross-reactivity. Abbreviations: glypican (GPC), human (Hu), mouse (Mo), strong (++), weak (+), none ( ). doi: https://doi.org/10.57733/addgene.r3dpk8

Immunofluorescence



Immunofluorescence (IF) of ExpiCHO cells transfected with human GPC1. ExpiCHO cells were transiently transfected with GFP (transfection control) and human GPC1. 100,000 cells were fixed to a 96-well glass bottom plate using 4% PFA at 37C for 15 min. Cells were permeabilized with 0.1% Triton X-100 at RT for 10 min and then blocked with 5% BSA, 5% serum, and 0.01% Triton X-100 at room temperature for 30 min. Cells were incubated with 2 µg/mL Anti-Glypican 1 (GPC1) [IPI-GPC1.44] (Addgene #222512). Alexa Fluor 647 F(ab')2 fragment goat anti-rabbit IgG, Fc fragment-specific (Jackson ImmunoResearch, 111-606-046) was used as the secondary antibody. Nuclear staining was performed using 0.5 mg/mL DAPI. Cells were imaged for GFP (green), DAPI (blue), and GPC1 (red) using an EVOS M7000 microscope at 10x magnification. doi: https://doi.org/10.57733/addgene.03a3kp

Antibody Details

Antibody design and production

Human variable domains for the heavy and light chain of the FAB fragment used in yeast display were grafted onto the constant CH1, CH2 and CH3 domains of rabbit IgG. The chimera human/rabbit IgG1 construct was recombinantly expressed in ExpiCHO cells, using pTipi2.1 as the expression vector. The antibody was purified by affinity chromatography using protein A (XYZ) and acid elution, followed by immediate buffer exchange using 1 x PBS buffer pH 7.4.

Sequence information

Heavy chain and light chain amino acid sequences are available upon request after purchase. **Contact us** to request.

Antibody Characterization

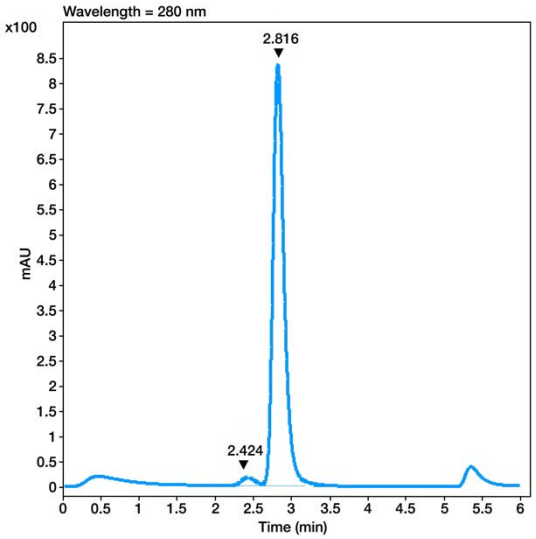
LC-MS: Intact mass analysis via LC-MS methods allows for confirmation antibody mass, and to identify any product-related variants such as glycosylation. Before conducting intact mass analysis via LC-MS, the antibody was reduced to its heavy chain (HC) and light chain (LC). This process allows for confirmation of the masses corresponding to the amino acid sequences of both chains.

	HC MW (Da) ***Calculated***	**HC MW (Da)** ***Observed***	**HC MW (Da) Delta**	**LC MW (Da)** ***Calculated***	**LC MW (Da)** ***Observed***	**LC MW (Da) Delta**
IPI-GPC1.44	50379.27	50383.70	4.43	22952.44	22952.02	-0.42

Heavy Chain (HC) Mass Calculation: The calculated molecular weight (MW) of the HC is derived by adding the mass of the unmodified HC amino acid sequence to the mass of the predominant N-glycan form (G0F), which is 1444.5 Da. This calculation assumes that the intrachain disulfide bonds remain intact. For HCs with an N-terminal glutamine (Q), the mass of Q is converted to pyroglutamic acid (PyroGlu), resulting in a deduction of 17.03 Da from the total mass. Additionally, for HCs with a C-terminal lysine (K), the mass of K (128.09 Da) is also subtracted.

Light Chain (LC) Mass Calculation: The calculated molecular weight (MW) of the LC is obtained from the mass of the unmodified LC amino acid sequence, assuming that the intrachain disulfide bonds are not reduced. For LCs with an N-terminal glutamine (Q), the mass of Q is converted to pyroglutamic acid (PyroGlu), leading to a deduction of 17.03 Da from the total mass. For LCs with a C-terminal lysine (K), the mass of K (128.09 Da) is subtracted as well.

Size Exclusion Chromatography (SEC): SEC is a protein purification technique that separates molecules based on size.



High-performance liquid chromatography-size exclusion chromatography (HPLC-SEC) profile. 5 µg of antibody (1 mg/mL) was analyzed. 1x PBS, pH 7.4 was used as the mobile phase. Protein standards ranging from 15-600 kDa were used for calibration (Sigma, 69385) and Lox1.9 antibody was run as an internal control. Resulting peaks were analyzed using Agilent OpenLab CDS software to determine total peak area and percentage purity.

	Purity	Result
IPI-GPC1.44	≥95%	Pass

Antigen Details

Immunogen design:

cDNA of Human Glypican 1 (GPC1) with C-terminal His-, Avi-, and HA-tags was produced in transiently transfected Expi293F cells and purified from culture supernatant by Ni-NTA affinity purification followed by size-exclusion chromatography.

Immunogen sequences:

Human GPC1 (AA: 23-497):
DPASKSRSCGEVRQIYGAKGFSLSDVPQAEISGEHLRICPQGYTCCTSEMEENLANRSHAELETALRDSS
RVLQAMLATQLRSFDDHFQHLLNDSERTLQATFPGAFGELYTQNARAFRDLYSELRLYYRGANLHLEETL
AEFWARLLERLFKQLHPQLLLPDDYLDCLGKQAEALRPFGEAPRELRLRATRAFVAARSFVQGLGVASD
VVRKVAQVPLGPECSRAVMKLVYCAHCLGVPGARPCPDYCRNVLKGCLANQADLDAEWRNLLDSMVLI
TDKFWGTSGVESVIGSVHTWLAEAINALQDNRDTLTAKVIQGCGNPKVNPQGPGPEEKRRRGKLAPRER
PPSGTLEKLVSEAKAQLRDVQDFWISLPGTLCSEKMALSTASDDRCWNGMARGRYLPEVMGDGLANQI
NNPEVEVDITKPDMTIRQQIMQLKIMTNRLRSAYNGNDVDFQDASDDGGSGHHHHHHHHHHGSGGLN
DIFEAQKIEWHEGSGYPYDVPDYA

Sequence information:

HUGO: 4449
Uniprot: P35052
Refseq: NM_002081.3

Structural information:

Topology: Glycosylphosphatidylinositol (GPI) Anchored
PDB IDs: 4BWE, 4YWT
Alphafold: AF-P35052-F1

Expression profiles:

Human Protein Atlas ENSG00000063660-GPC1

References

1. Z. Anderson, H. Li, T. Riedel, H Zhu, R. Meijers, and D. Moshinsky. (2024). Flow Cytometry for Anti-Glypican 1 (GPC1) [IPI-GPC1.44]. Addgene. https://doi.org/10.57733/addgene.r3dpk8
2. H. Li, Z. Anderson, T. Riedel, and D. Moshinsky. (2024). IF for Anti-Glypican 1 (GPC1) [IPI-GPC1.44]. Addgene. https://doi.org/10.57733/addgene.03a3kp
3. A. Salic. (2024). Immunofluorescence for Anti-glypican 1 (GPC1) [IPI-GPC1.44]. Addgene. https://doi.org/10.57733/addgene.4203q1

How to cite this antibody:
Anti-Glypican 1 (GPC1) [IPI-GPC1.44] - from Institute for Protein Innovation (IPI) (Addgene #222512; http://n2t.net/addgene:222512; RRID: AB_3101859).

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