

## Product Datasheet

### [Anti-Glypican 6 \(GPC6\) \[IPI-GPC6.6\]](#)

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#### Overview

Antigen	Glypican 6 (GPC6)
Immunogen	Purified recombinant fragment of Human Glypican 6 (GPC6), corresponding to AA: 24-536.
Host/isotype	Rabbit/IgG
Clonality	Recombinant monoclonal
Clone name	IPI-GPC6.6
RRID	AB_3697525
IPI ID	TAB0009600-013-002
Specificity	GPC6; Does not recognize other GPCs
Species reactivity	Human and mouse
Amount	100 µg
Concentration	1 mg/mL
Purification	Expressed in HEK293 cells and affinity purified using Protein A
Storage buffer	PBS, pH 7.4
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<sup>‡</sup>

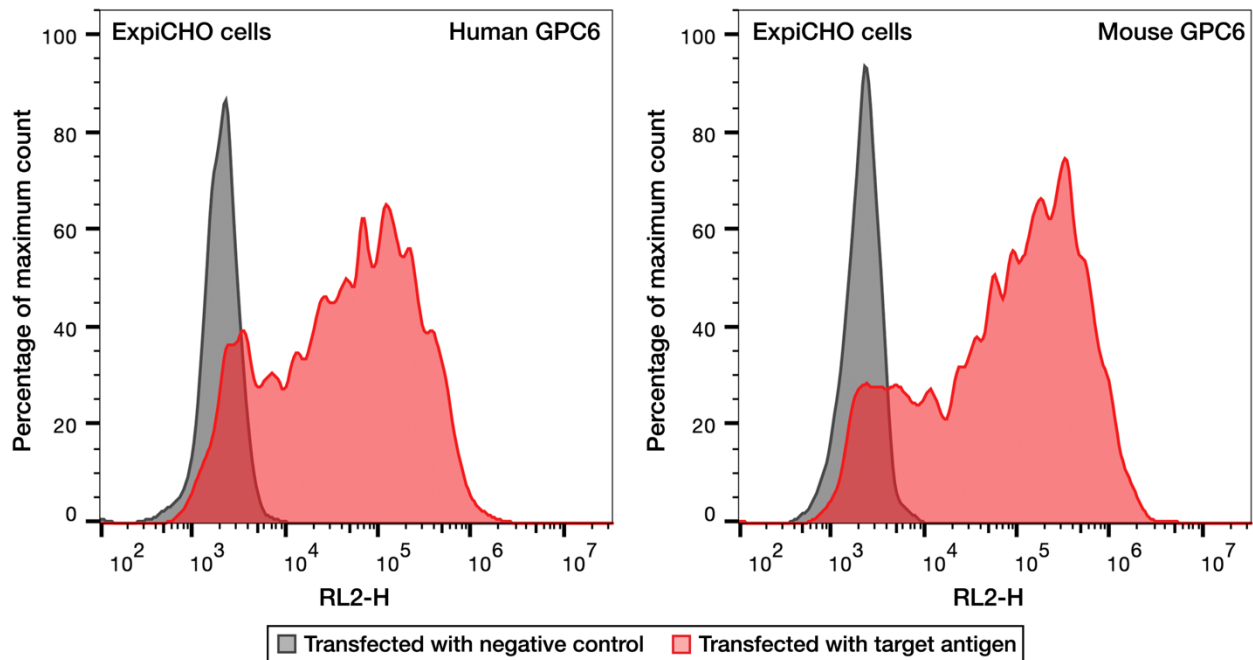
Application	Tested concentration	Result	Reference
Flow	0.66-100 µg/mL	Positive	<a href="https://doi.org/10.57733/addgene.4v9lgn">https://doi.org/10.57733/addgene.4v9lgn</a>
IF – Binding	1 µg/mL	Positive	<a href="https://doi.org/10.57733/addgene.5pok37">https://doi.org/10.57733/addgene.5pok37</a>
IF – Specificity	1 µg/mL	Positive	<a href="https://doi.org/10.57733/addgene.bx02yk">https://doi.org/10.57733/addgene.bx02yk</a>
IF – Endogenous	5 µg/mL	Positive	<a href="https://doi.org/10.57733/addgene.w58ubp">https://doi.org/10.57733/addgene.w58ubp</a>

<sup>‡</sup> Not suitable for WB application.

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## Applications

### Flow cytometry

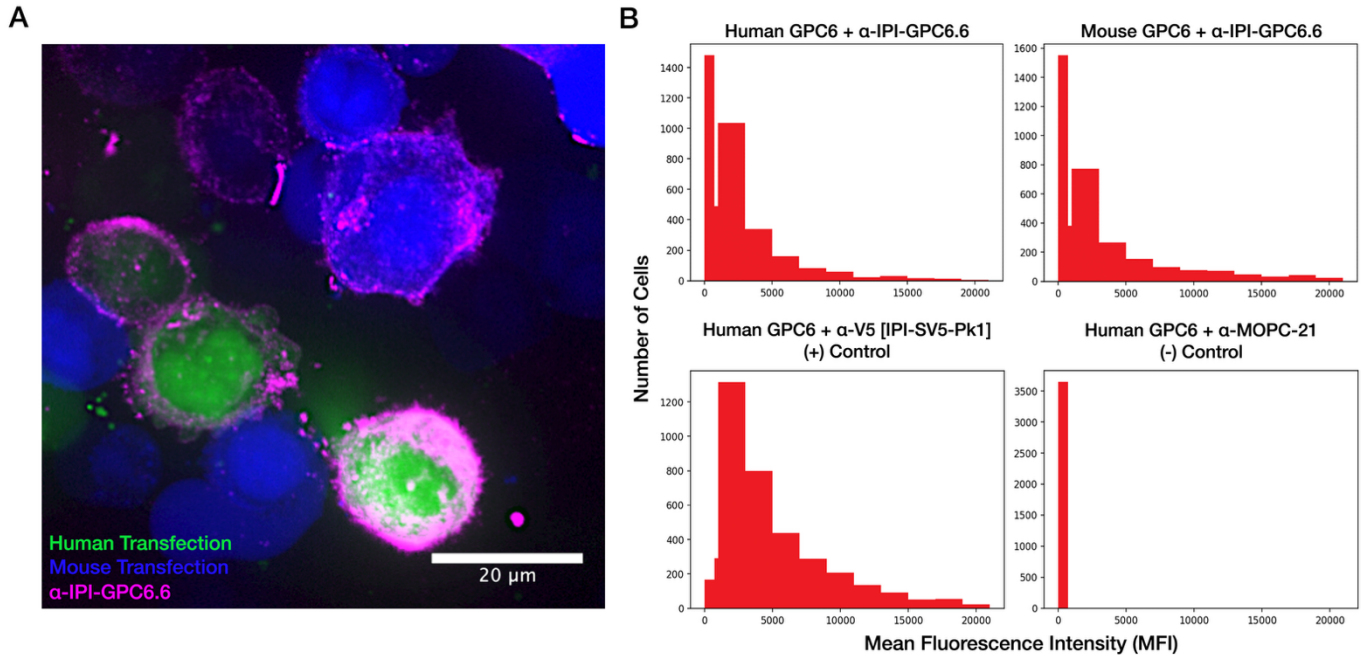


**Anti-Glypican 6 (GPC6) [IPI-GPC6.6] (Addgene #241001) recognizes human and mouse GPC6 in flow cytometry.** Histogram from FACS analysis on ExpiCHO cells transfected with human or mouse GPC6 (red), or B7H3 negative control (gray). Cells expressing human (left panel) or mouse (right panel) GPC6 were labeled with Anti-Glypican 6 (GPC6) [IPI-GPC6.6] and Alexa Fluor 647 F(ab')<sub>2</sub> goat anti-rabbit IgG Fc fragment (Jackson ImmunoResearch, 111-606-046). Labeled cells were analyzed with an Intellicyt iQue Screener Plus flow cytometer. Histograms were generated and normalized to mode using FlowJo™ v10.10. doi: <https://doi.org/10.57733/addgene.4v9lgn>

**EC<sub>50</sub> (data not shown):** A fourteen-point titration of antibody concentrations, ranging from 660 nM (0.1 mg/mL) to 4.42 pM with a 1:2.5 dilution factor, against human and mouse GPC6 showed reactivity towards human and mouse GPC6 with observed EC<sub>50</sub> values of 0.81 nM and 0.56 nM for human and mouse GPC6, respectively.

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## Immunofluorescence (IF) – Species Reactivity



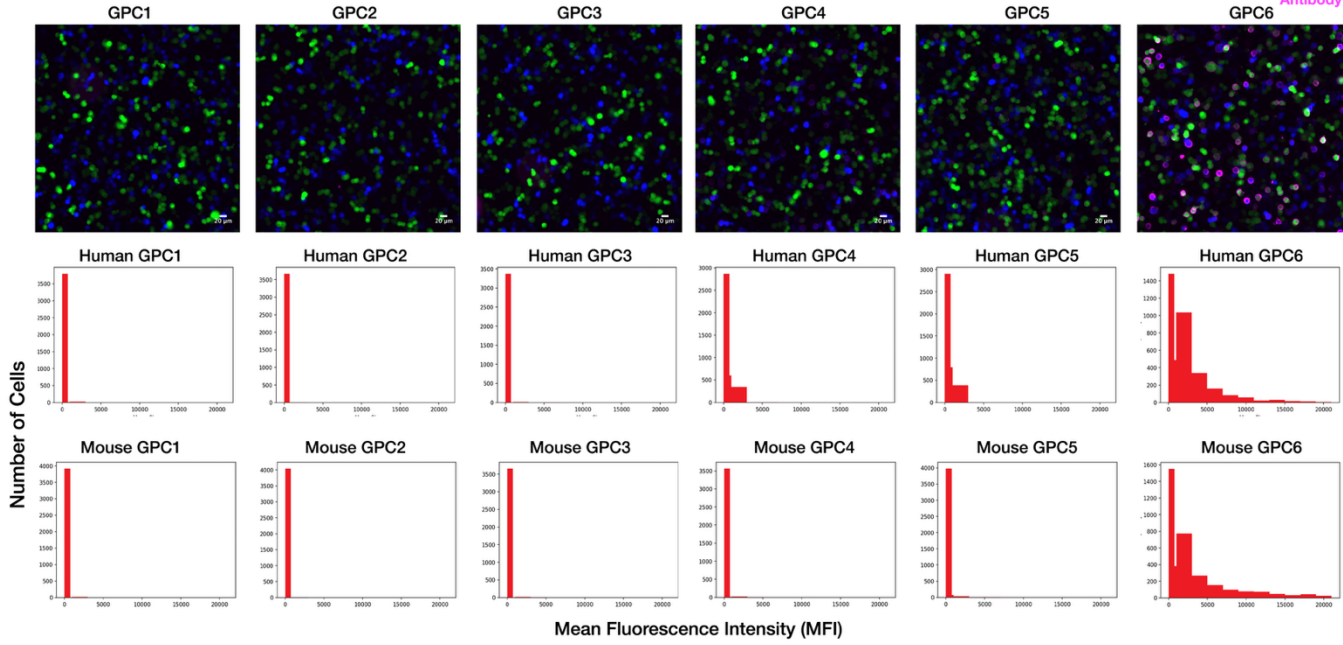
**Anti-Glypican 6 (GPC6) [IPI-GPC6.6] (Addgene #241001) recognizes human and mouse GPC6 in immunofluorescence.** A) Immunofluorescence (IF) of ExpiCHO cells transfected with human and mouse GPC6. Human GPC6 was co-transfected with GFP (human transfection control) and mouse GPC6 was co-transfected with BFP (mouse transfection control). Confocal images taken at 40X magnification on the ImageXpress confocal HT.ai microscope. Cells were imaged for GFP (green), BFP (blue), and GPC6 (magenta). B) Combined quantification of multiple images of the same transfected cells taken at 10X magnification. GFP- or BFP- positive cells were identified using a custom module in the IN Carta image analysis software, then the mean fluorescence intensity (MFI) of the far-red channel for each cell, representing IPI-GPC6.6 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 450 (background fluorescence) to 20000. IPI-GPC6.6 staining of human (left) and mouse (right) GPC6 are shown in the top row, and compared to a positive (left) and negative (right) control in the bottom row. For both panels, IPI-GPC6.6 was used at 1 µg/mL (1:1,000 dilution). doi:

<https://doi.org/10.57733/addgene.5pok37>

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**Immunofluorescence (IF) – Target Specificity**

Human Transfection  
Mouse Transfection  
Antibody

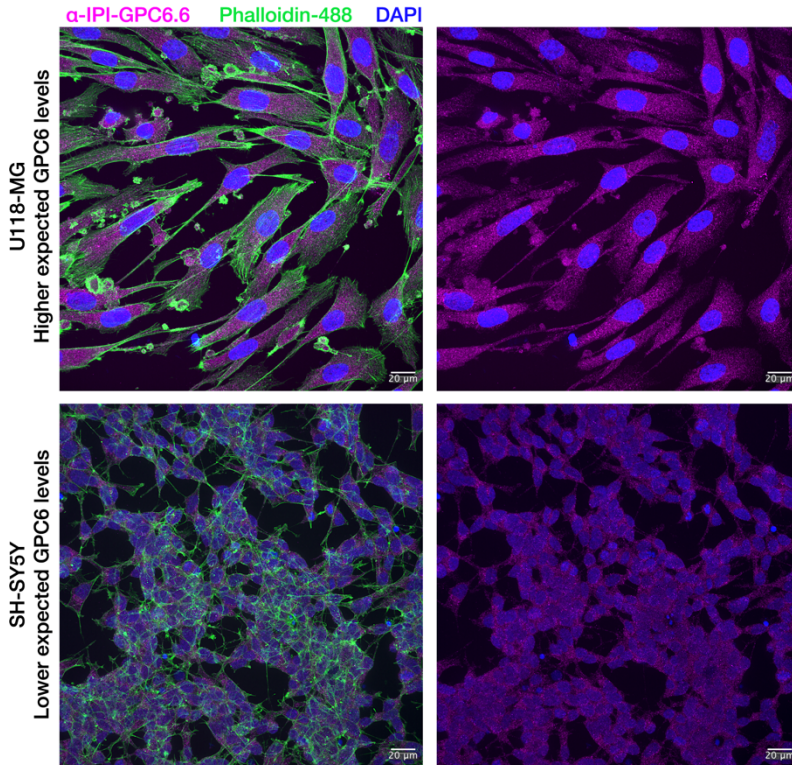


		GPC Specificity							
		GPC1	GPC2	GPC3	GPC4	GPC5	GPC6	Strong	++
		Hu	Mo	Hu	Mo	Hu	Mo	Hu	Mo
IPI-GPC6.6								++	++
								+	+
								None	None

**Anti-Glypican 6 (GPC6) [IPI-GPC6.6] (Addgene #241001) is specific for GPC6 in immunofluorescence.** (Top) Immunofluorescence (IF) of ExpiCHO cells transfected with human and mouse GPC1-GPC6. Human GPC targets were co-transfected with GFP (human transfection control) and mouse GPC targets were co-transfected with BFP (mouse transfection control). Widefield images were taken at 10X magnification on an imageXpress confocal HT.ai microscope. (Bottom) Each graph depicts the combined quantification of multiple images of the same transfected cells taken at 10X magnification. GFP- or BFP- positive cells were identified using a custom module in the InCarta software, then the mean fluorescence intensity (MFI) of the far-red channel for each cell, representing IPI-GPC6.6 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 450 (background fluorescence) to 20000. IPI-GPC6.6 staining of human and mouse variants of each GPC family member is compared on the top and bottom rows. For all panels, IPI-GPC6.6 was used at 1 µg/mL (1:1,000 dilution). doi: <https://doi.org/10.57733/addgene.bx02yk>

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### Immunofluorescence (IF) – Endogenous



**Anti-Glypican 6 (GPC6) [IPI-GPC6.6] (Addgene #241001) is recognizes endogenous GPC6 in immunofluorescence.** Immunofluorescence (IF) of U118-MG cells (top row) and SH-SY-5Y cells (bottom row) stained for GPC6 using IPI-GPC6.6 and for actin with Alexa Fluor 488-conjugated phalloidin. Confocal images taken at 40X magnification on an ImageXpress HT confocal.ai microscope. Left images show all 3 channels (IPI-GPC6.6, actin, and DAPI) while the right images only show IPI-GPC6.6 and DAPI. U118-MG cells are predicted to express GPC6 highly and SH-SY-5Y cells are predicted to not express GPC6 highly based on publicly available data (DepMap.org). For both panels, IPI-GPC6.6 was used at 5 µg/mL (1:200 dilution). doi: <https://doi.org/10.57733/addgene.w58ubp>

## **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 Expi HEK293 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.

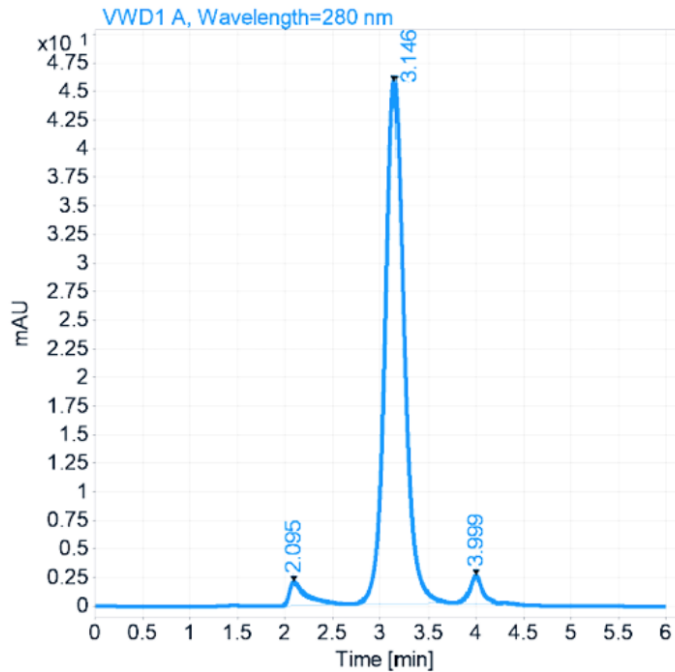
	<b>HC MW (Da) <i>Calculated</i></b>	<b>HC MW (Da) <i>Observed</i></b>	<b>HC MW (Da) <i>Delta</i></b>	<b>LC MW (Da) <i>Calculated</i></b>	<b>LC MW (Da) <i>Observed</i></b>	<b>LC MW (Da) <i>Delta</i></b>
<b>IPI-GPC6.6</b>	49646.47	49651.30	4.83	22952.44	22952.04	-0.40

**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.

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



	RT (min)	Width (min)	Area	Height	Area %
<b>IPI-GPC6.6</b>	3.146	0.2011	606.6708	45.6305	92.2777

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## **Antigen Details**

### **Immunogen design:**

cDNA of Human Glypican 6 (GPC6) with C-terminal His-, Avi-, and FLAG-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 GPC6 (AA: 24-536):

DVKARSCGEVRQAYGAKGFSLADIPYQEIAGEHLRIPCQEYTCCTTEMEDKLSQQSKLEFENLVEETSHF  
VRTTFVSRHKKFDEFFRELLENAEKSLNDMFVRTYGMPLYMQNSEVFQDLFTELKRYTGGNVNLEEMLN  
DFWARLLERMFLINPQYHFSEDYLECVSKYTDQLKPFQDVPRLKIQVTRAFIAARTFVQGLTVGREVA  
NRVSKVSPTPGCIRALMKMLYCPYCRGLPTVRPCNNYCLNVMKGCLANQADLDTEWNLFDAMLLVAE  
RLEGPFNIESVMDPIDVKISEAIMNMQENSMQVSAKFQCGQPKPAPALRSARSAPENFNTRFRPYNP  
EERPTTAAGTSLDRLVTDIKEKLSKKVWSALPYTICKDESVTAGTSNEEECWNGHSHKARYLPEIMNDG  
LTNQINNPEVDVDITRPDTFIRQQIMALRVMTNKLKNAYNGNDVNFQDTSDESSGSGSGSGCMDDVCPT  
EFEFVTTEAPAVDPDRREVDGSGHHHHHHHHHHGSGDYKDDDDKSGGGLNDIFEAQKIEWHE

### **Sequence information:**

HUGO: 4454  
Uniprot: Q9Y625  
Refseq: NM\_005708.5

### **Structural information:**

Topology: Glycosylphosphatidylinositol (GPI) Anchored  
PDB IDs: -  
AlphaFold: AF-Q9Y625-F1

### **Expression profiles:**

Human Protein Atlas ENSG00000183098-GPC6

**How to cite this antibody:**

Anti-Glypican 6 (GPC6) [IPI-GPC6.6] - from Institute for Protein Innovation (IPI) (Addgene #241001; <http://n2t.net/addgene:241001>; RRID: AB\_3697525).

If you publish research with this product, please [let us know](#) so that we can cite your paper.

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