Product Datasheet

Anti-Glypican 4 (GPC4) [IPI-mGPC4.3]

Applications | Antibody Details | Antigen Details | References

Overview

Antigen	Glypican 4 (GPC4)
Immunogen	Purified recombinant fragment of Mouse Glypican 4 (GPC4), corresponding to AA: 23-557.
Host/isotype	Rabbit/IgG
Clonality	Recombinant monoclonal
Clone name	IPI-mGPC4.3
RRID	AB_3697523
IPI ID	TAB0010812-013-002
Specificity	GPC4; Does not recognize other GPCs
Species reactivity	Mouse and human
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‡

Application	Tested concentration	Result	Reference
Flow	0.66-100 µg/mL	Positive	https://doi.org/10.57733/addgene.2qfs6s
IF – Binding	1 µg/mL	Positive	https://doi.org/10.57733/addgene.fst0c5
IF – Specificity	1 µg/mL	Positive	https://doi.org/10.57733/addgene.orgles
IF – Endogenous	5 µg/mL	Positive	https://doi.org/10.57733/addgene.l9w2oa

‡ Not suitable for WB application.

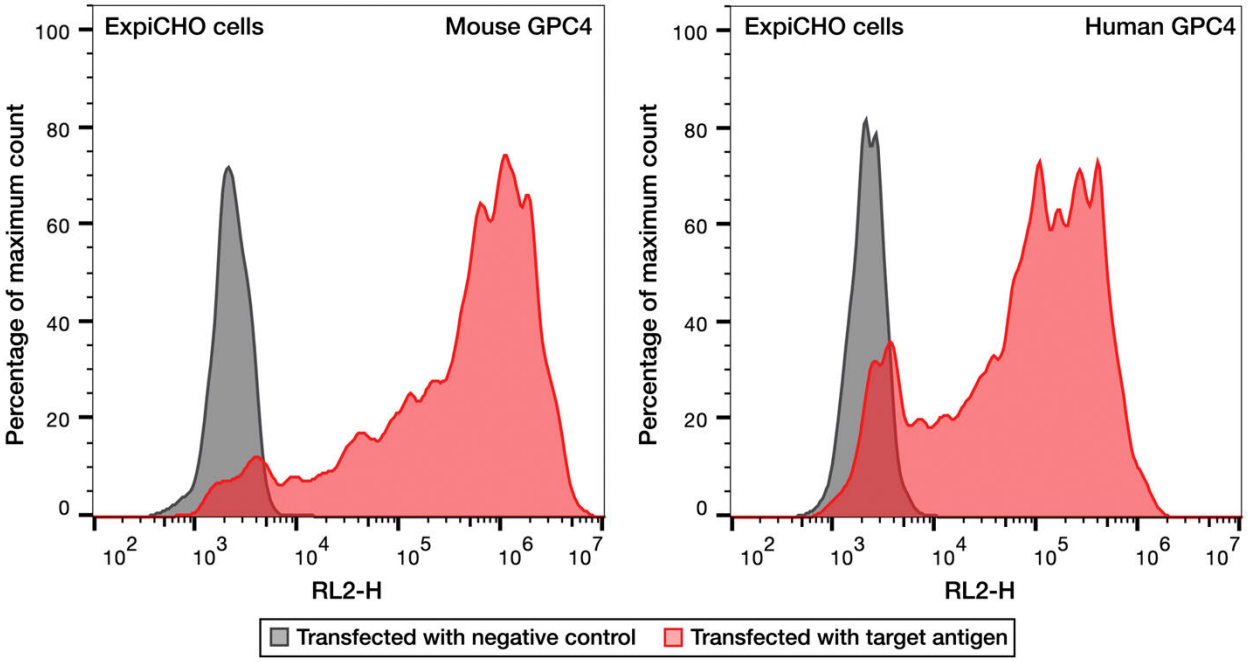
Community Data*

Application	Lab	Reference
IHC	James Trimmer, Ph.D., UC Davis/NeuroMab	https://doi.org/10.57733/addgene.92oupa

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

Applications

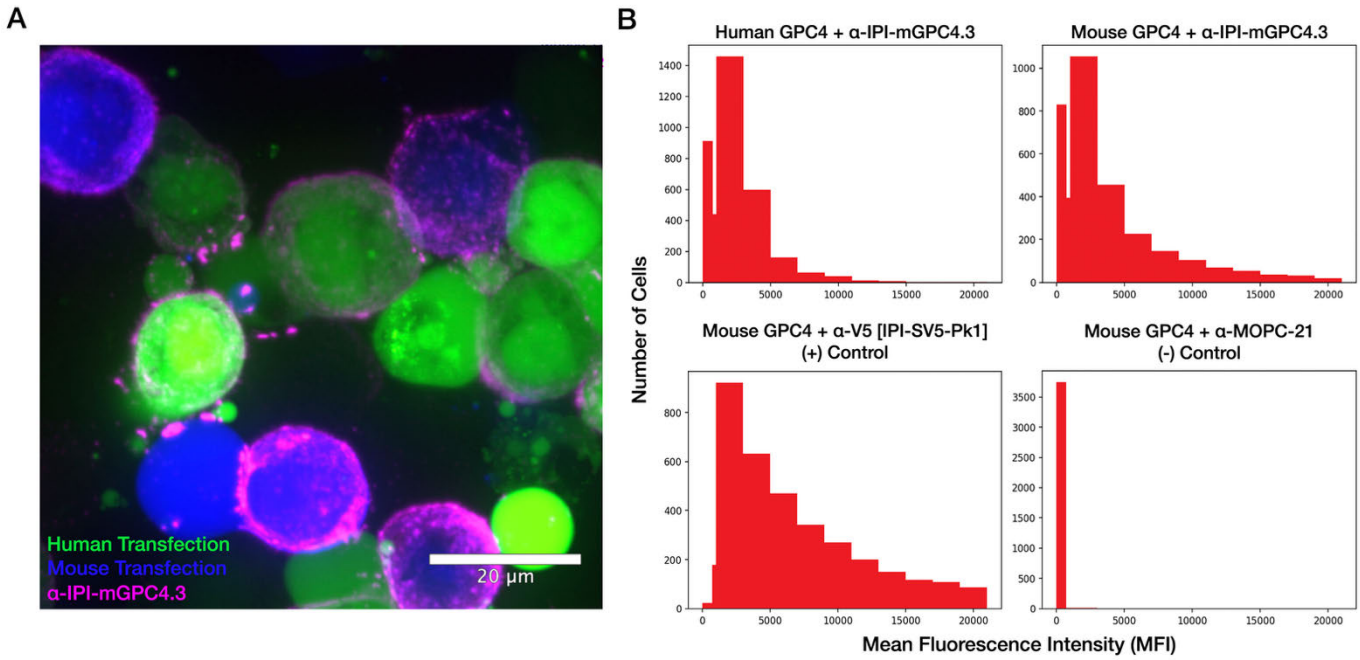
Flow cytometry



Anti-Glypican 4 (GPC4) [IPI-mGPC4.3] (Addgene #240999) recognizes mouse and human GPC4 in flow cytometry. Histogram from FACS analysis on ExpiCHO cells transfected with mouse or human GPC4 (red), or B7H3 negative control (gray). Cells expressing mouse (left panel) or human (right panel) GPC4 were labeled with Anti-Glypican 4 (GPC4) [IPI-mGPC4.3] and Alexa Fluor 647 F(ab')2 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.2qfs6s

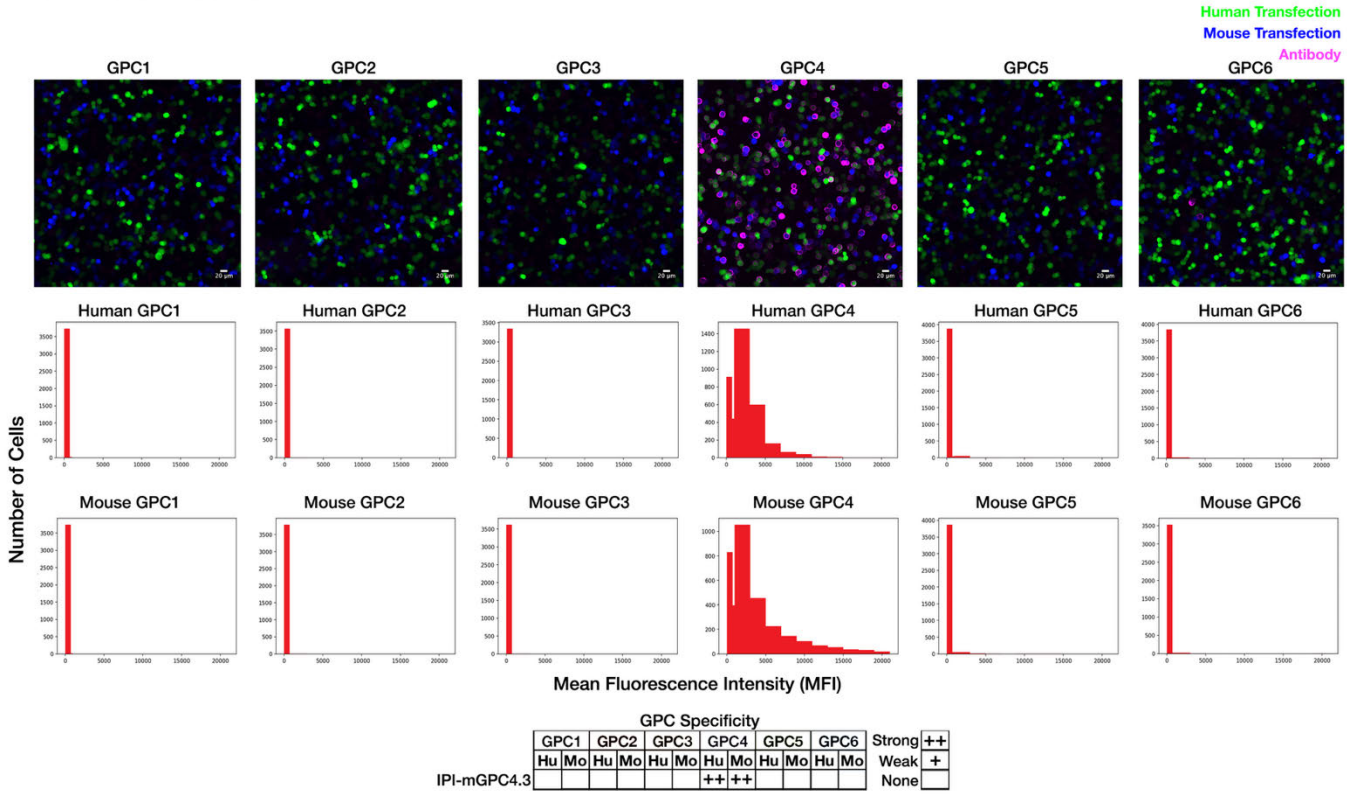
EC_{50} (data not shown): A fourteen-point titration of antibody concentrations, ranging from 660 nM (0.1 mg/mL) to 4.42 pM with a 1:2.5 dilution factor, against human and mouse GPC4 showed reactivity towards human and mouse GPC4 with observed EC_{50} values of 0.41 nM and 0.21 nM for human and mouse GPC4, respectively.

Immunofluorescence (IF) – Species Reactivity



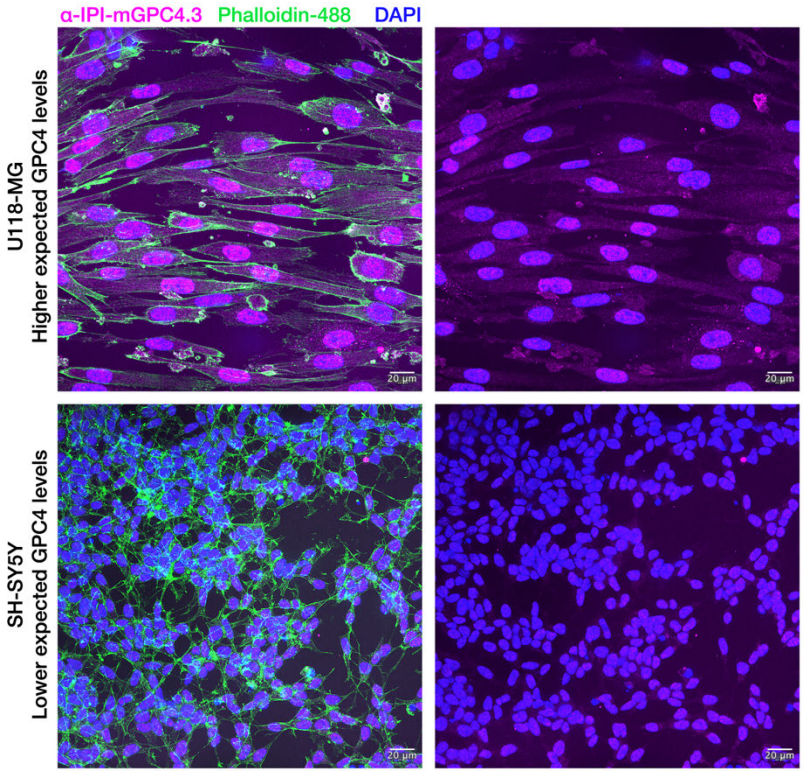
Anti-Glypican 4 (GPC4) [IPI-mGPC4.3] (Addgene #240999) recognizes mouse and human GPC4 in immunofluorescence. A) Immunofluorescence (IF) of ExpiCHO cells transfected with human and mouse GPC4. Human GPC4 was co-transfected with GFP (human transfection control) and mouse GPC4 was co-transfected with BFP (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 GPC4 (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-mGPC4.3 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 450 (background fluorescence) to 20000. IPI-mGPC4.3 staining of human (left) and mouse (right) GPC4 are shown in the top row, and compared to a positive (left) and negative (right) control in the bottom row. For both panels, IPI-mGPC4.3 was used at 1 µg/mL (1:1,000 dilution). doi: https://doi.org/10.57733/addgene.fst0c5

Immunofluorescence (IF) – Target Specificity



Anti-Glypican 4 (GPC4) [IPI-mGPC4.3] (Addgene #240999) is specific for GPC4 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-mGPC4.3 staining, was recorded. Each histogram displays the number of cells with MFIs ranging from below 450 (background fluorescence) to 20000. IPI-mGPC4.3 staining of human and mouse variants of each GPC family member is compared on the top and bottom rows. For all panels, IPI-mGPC4.3 was used at 1 µg/mL (1:1,000 dilution). doi: https://doi.org/10.57733/addgene.orgles

Immunofluorescence (IF) – Endogenous



Anti-Glypican 4 (GPC4) [IPI-mGPC4.3] (Addgene #240999) recognizes endogenous GPC4 in immunofluorescence. Immunofluorescence (IF) of U118-MG cells (top row) and SH-SY-5Y cells (bottom row) stained for GPC4 using IPI-mGPC4.3 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-mGPC4.3, actin, and DAPI) while the right images only show IPI-mGPC4.3 and DAPI. U118-MG cells are predicted to express GPC4 highly and SH-SY-5Y cells are predicted to not express GPC4 highly based on publicly available data (DepMap.org). For both panels, IPI-mGPC4.3 was used at 5 µg/mL (1:200 dilution). doi: https://doi.org/10.57733/addgene.l9w2oa

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

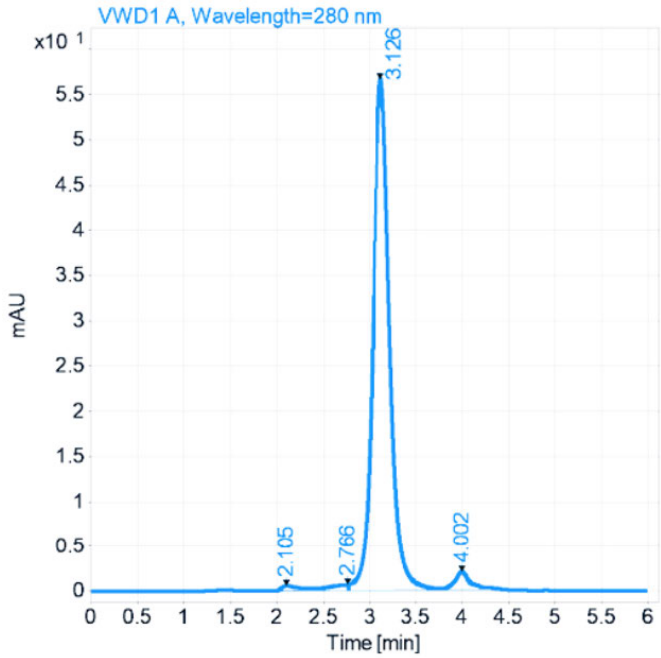
LC-MS: Intact mass analysis via LC-MS methods allows for confirmation antibody mass, and to identify any product-related variants such as glycosylation. Before conducting intact mass analysis via LC-MS, the antibody was reduced to its heavy chain (HC) and light chain (LC). This process allows for confirmation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

	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-mGPC4.3	50088.05	50091.30	3.25	22952.44	22951.85	-0.59

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.

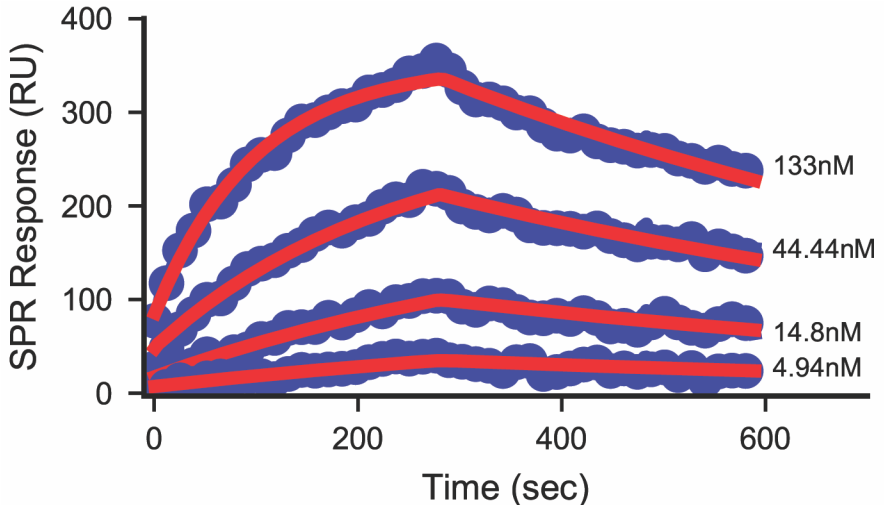
Light Chain (LC) Mass Calculation: The calculated molecular weight (MW) of the LC is obtained from the mass of the unmodified LC amino acid sequence, assuming that the intrachain disulfide bonds are not reduced. For LCs with an N-terminal glutamine (Q), the mass of Q is converted to pyroglutamic acid (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.



	RT (min)	Width (min)	Area	Height	Area %	Result
IPI-mGPC4.3	3.126	0.2145	729.0434	56.6368	94.34	Pass

Surface Plasmon Resonance (SPR)



	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	Res. sd
IPI-mGPC4.3	6.5×10^{4}	1.3×10^{-3}	2.0×10^{-8}	6.3

Surface Plasmon Resonance (SPR) kinetics analysis of the interaction between Anti-GPC4 [IPI-mGPC4.3] and mouse GPC4. SPR binding kinetics were measured on a Carterra LSA using HC30M chips (Carterra, cat. #4279) at 25 °C. Goat anti-rabbit IgG Fc (Jackson ImmunoResearch, cat. #111-005-046) was immobilized via amine coupling, and test antibodies were captured using a 96-channel print-head. Antigens (400 nM to five lower concentrations, 2-fold dilutions) were injected in antigen buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.005% Tween 80) with 300 s association/dissociation phases and acid regeneration. Data (reference/buffer subtracted, smoothed) were globally fit to a 1:1 Langmuir model to derive k_a, k_d, and K_D using Carterra Kinetics software v1.9.2.44.63, and replotted in OriginPro 2023b. Results show a high-affinity and specific binding event between the antibody and antigen.

Antigen Details

Immunogen design:

cDNA of Mouse Glypican 4 (GPC4) with C-terminal His- 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:

>Mouse GPC4 (AA: 23-557):
AELKSKSCSEVRRLYVSKGFNKNDAPLYEINGDHLKICPQDYTCCSQEMEEKYSLQSKDDFKTVVSEQC
NHLQAIFASRYKKFDEFFKELLENAEKSLNDMFVKTYGHLYMQNSELFKDLFVELKRYYVAGNVNLEEML
NDFWARLLERMFRLVNSQYHFTDEYLECVSKYTEQLKPFGDVPRKLKLQVTRAFVAARTFAQGLAVARD
VVSKVSVVNPTAQCTHALLKMIYCSHCRGLVTVKPCYNYCSNIMRGCLANQGDLDFEWNNFIDAMLMV
AERLEGPFNIESVMDPIDVKISDAIMNMQDNSVQVSQKVFQGCGPPKPLPAGRISRSISESAFSARFRPY
HPEQRPTTAAGTSLDRLVTDVKEKLKQAKKFWSSLPSTVCNDERMAAGNENEDDCWNGKGKSRYLFA
VTGNGLANQGNNPEVQVDTSKPDILILRQIMALRVMTSKMKNAYNGNDVDFFDISDESSGEGSGSGCEY
QQCPSEFEYNATDHSGKSANEKADSGSGHHHHHHHHHHGSGGLNDIFEAQKIEWHEGSGDYKDDDD
K

Sequence information:

HUGO:	MGI:104902
Uniprot:	P51655
Refseq:	NM_008150.2

Structural information:

Topology:	Glycosylphosphatidylinositol (GPI) Anchored
PDB IDs:	-
Alphafold:	AF-P51655-F1

Expression profiles:

Human Protein Atlas ENSMUSG00000031119

References

1. J. Trimmer. (2025). Anti-Glypican 4 (GPC4) [IPI-mGPC4.3] in Immunohistochemistry (Rat). Addgene. https://doi.org/10.57733/addgene.92oupa

How to cite this antibody:
Anti-Glypican 4 (GPC4) [IPI-mGPC4.3] - from Institute for Protein Innovation (IPI) (Addgene #240999; http://n2t.net/addgene:240999; RRID: AB_3697523).

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