Product Datasheet

Glut4 Antibody - BSA Free

NBP1-49533

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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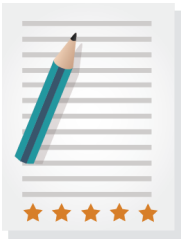
Updated 11/12/2024 v.20.1



NBP1-49533

Glut4 Antibody - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description

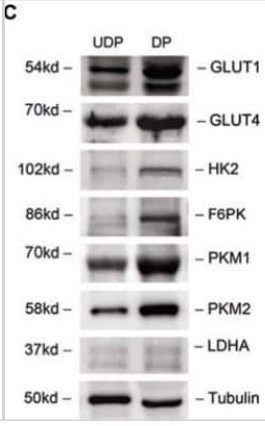
Host	Rabbit
Gene ID	6517
Gene Symbol	SLC2A4
Species	Human, Mouse, Rat, Plant
Reactivity Notes	Plant reactivity reported in scientific literature (PMID: 31555030).
Immunogen	A synthetic peptide from the human Glucose Transporter GLUT4 protein [UniProt P14672]

Product Application Details

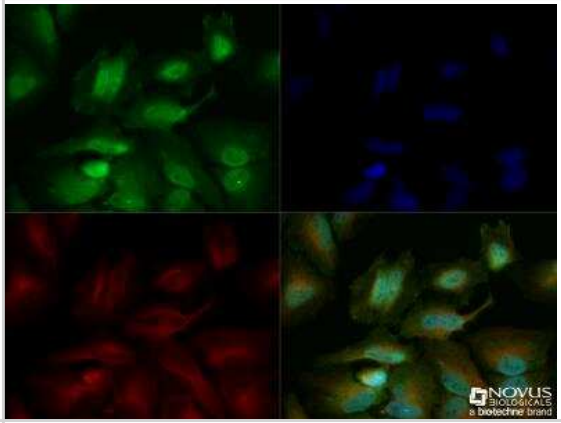
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Knockdown Validated
Recommended Dilutions	Western Blot 0.5ug/ml, Flow Cytometry reported by customer review, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:100, Immunohistochemistry-Paraffin reported in scientific literature (PMID 24339864), Flow (Intracellular), Knockdown Validated reported in scientific literature (PMID 31555030)

Images

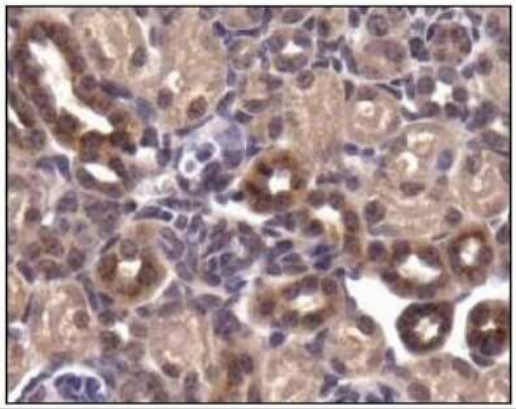
Western Blot: Glut4 Antibody [NBP1-49533] - Glycolysis-related genes and proteins were upregulated in differentiated podocytes. Representative blot images of glycolysis-related proteins (n =3). d All proteins were normalized to tubulin and compared to UDPs. *P < 0.05, **P < 0.01, determined by t test. Data are shown as the means +/- SD. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41419-020-2481-5), licensed under a CC-BY license.



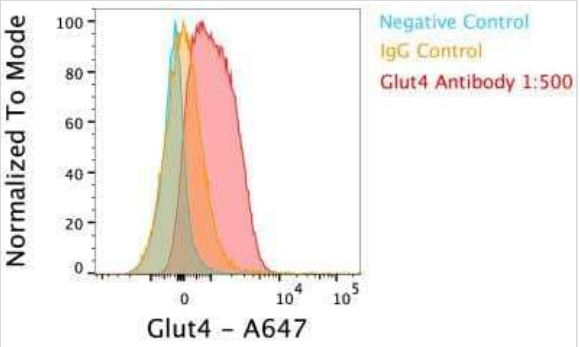
Immunocytochemistry/Immunofluorescence: Glut4 Antibody [NBP1-49533] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-GLUT4 [NBP1-49533] at a 1:200 dilution overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse DyLight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



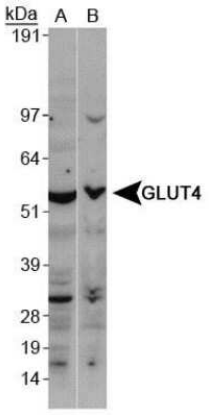
Immunohistochemistry: Glut4 Antibody [NBP1-49533] - Analysis of GLUT4 in mouse kidney

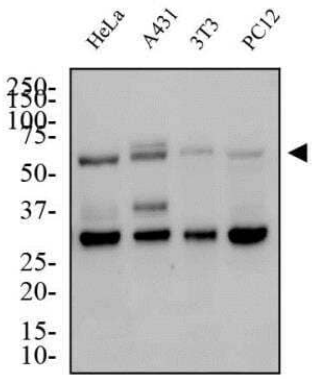
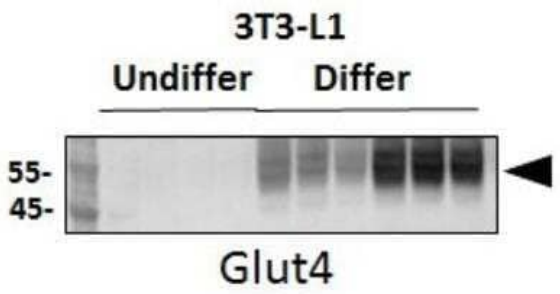
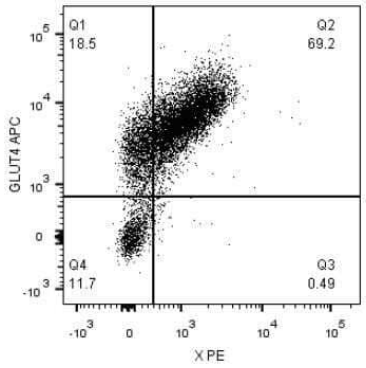
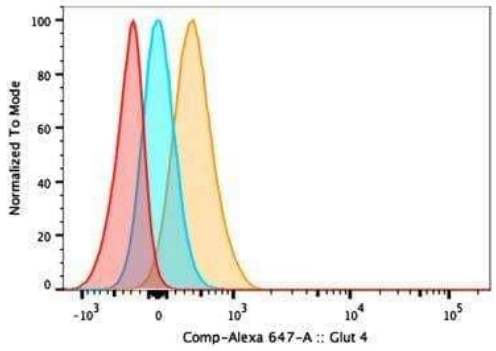


Flow Cytometry: Glut4 Antibody - BSA Free [NBP1-49533] - Analysis using the Alexa Fluor (R) 647 conjugate of NBP1-49533 (NBP1-49533AF647) on mouse Thymic Epithelial cells. Glut4 antibody in comparison to negative and IgG Control. Primary antibody dilution: 1:500. Image from verified customer review.

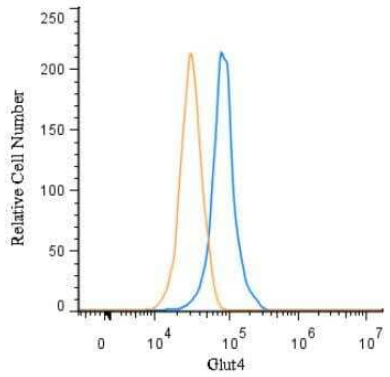


Western Blot: Glut4 Antibody [NBP1-49533] - Analysis of GLUT4 in A) MCF7 whole cell lysate and B) 3T3L1 whole cell lysate.



Western Blot: Glut4 Antibody [NBP1-49533] - Total protein from Human HeLa and A431, Mouse 3T3 and Rat PC12 cells was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-Glut4 in 1% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.	
Western Blot: Glut4 Antibody [NBP1-49533] - Total protein from 3T3-L1 mouse embryonic fibroblast adipose-like cell line, separated by 4-12% SDS-PAGE, transferred to nitrocellulose membrane and blocked in 5% non-fat milk for 1h at room temperature. The membrane was probed with anti-Glut4 0.5 ug/ml in non-fat milk. Undiffer: undifferentiated; Differ: Differentiated. Image from verified customer review.	
Flow Cytometry: Glut4 Antibody [NBP1-49533] - GLUT4 Biotin/APC vs. [X] PE. Image from verified customer review.	
Flow Cytometry: Glut4 Antibody [NBP1-49533] - Analysis using the Alexa Fluor (R) 647 conjugate of NBP1-49533. Staining of Glut 4 expression on Murine CD4+ T cells stimulated with anti-CD3/CD28 beads and insulin (1ug/mL) for 5 days in culture media with additional glucose provided. This Alexa Fluor (R) 647 conjugated Glut 4 antibody (orange) positively stained mouse CD4+ T cells compared to Isotype Control (Rb IgG AF647, Novus NBP2-36463AF647, blue) and fluorescence minus one/FMO control (red). Image from verified customer review.	

Flow (Intracellular): Glut4 Antibody [NBP1-49533] - An intracellular stain was performed on HepG2 with NBP1-49533 and a matched isotype control. Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody.



Publications

Supruniuk E, Miklosz A, Chabowski A Pyrroloquinoline Quinone Modifies Lipid Profile, but Not Insulin Sensitivity, of Palmitic Acid-Treated L6 Myotubes Nat Commun 2020-11-05 [PMID: 33171690]

Allison Campolo, Zahra Maria, Véronique A. Lacombe, Victor Gault Diabetes Causes Significant Alterations in Pulmonary Glucose Transporter Expression Metabolites 2024-05-07 [PMID: 38786744]

BL Phipps, U Suwannasua, J Lucero, NA Mitchell, AK Lund Vehicle emissions-exposure alters expression of systemic and tissue-specific components of the renin-angiotensin system and promotes outcomes associated with cardiovascular disease and obesity in wild-type C57BL/6 male mice Toxicology reports, 2021-04-15;8(0):846-862. 2021-04-15 [PMID: 33948438]

Fabio Verginelli, Silvia Perconti, Simone Vespa, Francesca Schiavi, Sampath Chandra Prasad, Paola Lanuti, Alessandro Cama, Lorenzo Tramontana, Diana Liberata Esposito, Simone Guarnieri, Artenca Sheu, Mattia Russel Pantalone, Rosalba Florio, Annalisa Morgano, Cosmo Rossi, Giuseppina Bologna, Marco Marchisio, Andrea D'Argenio, Elisa Taschin, Rosa Visone, Giuseppe Opocher, Angelo Veronese, Carlo T. Paties, Vinagolu K. Rajasekhar, Cecilia Söderberg-Nauclér, Mario Sanna, Lavinia Vittoria Lotti, Renato Mariani-Costantini Paragangliomas arise through an autonomous vasculo-angio-neurogenic program inhibited by imatinib Acta Neuropathologica 2018-01-05 [PMID: 29305721]

Ning Hou, Yunpei Mai, Xiaoxia Qiu, Wenchang Yuan, Yilang Li, Chengfeng Luo, Yun Liu, Guiping Zhang, Ganjiang Zhao, Jian-dong Luo Carvacrol Attenuates Diabetic Cardiomyopathy by Modulating the PI3K/AKT/GLUT4 Pathway in Diabetic Mice Frontiers in Pharmacology 2019-09-12 [PMID: 31572181]

Challa NL, Sarkar A, Kapettu S et al. TGS1/PIMT regulates pro-inflammatory macrophage mediated paracrine insulin resistance: Crosstalk between macrophages and skeletal muscle cells Biochimica et biophysica acta. Molecular basis of disease 2023-09-04 [PMID: 37673359] (WB, Mouse)

Molina-Fern□ndez R, Pic□n-Pag□s P, Barranco-Almohalla A et al. Differential regulation of insulin signalling by monomeric and oligomeric amyloid beta-peptide Brain Communications 2022-09-24 [PMID: 36267327] (Immunocytochemistry/ Immunofluorescence)

Takase K, Kakuta I Oral administration of wild plant-derived minerals and red ginseng ameliorates insulin resistance in fish through different pathways Physiological reports 2023-04-01 [PMID: 37078367] (WB, Fish)

Kohler ZM, Trencsenyi G, Juhasz L et al. Tilorone increases glucose uptake in vivo and in skeletal muscle cells by enhancing Akt2/AS160 signaling and glucose transporter levels Journal of cellular physiology 2023-04-03 [PMID: 37012691] (Western Blot, Mouse)

Muñoz VR, Botezelli JD, Gaspar RC et al. Effects of short-term endurance and strength exercise in the molecular regulation of skeletal muscle in hyperinsulinemic and hyperglycemic Slc2a4+/- mice Cellular and molecular life sciences : CMLS 2023-04-13 [PMID: 37052684]

Kapadia B, Behera S, Kumar ST et al. PIMT regulates hepatic gluconeogenesis in mice iScience 2023-03-17 [PMID: 36866247]

Ladraa S, Zerbib L, Bayard C et al. PIK3CA gain-of-function mutation in adipose tissue induces metabolic reprogramming with Warburg-like effect and severe endocrine disruption Science advances 2022-12-09 [PMID: 36490341] (FLOW, Mouse)

More publications at http://www.novusbio.com/NBP1-49533

Procedures

Immunohistochemistry-Paraffin protocol for Glucose Transporter GLUT4 Antibody (NBP1-49533)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

Immunocytochemistry/ Immunofluorescence Protocol for Glut4 Antibody (NBP1-49533)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Western Blot protocol for Glut4 Antibody (NBP1-49533)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.

Flow (Intracellular) Protocol for Glut4 Antibody (NBP1-49533)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.

a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.

4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 1 mL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeablization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

Optional: Perform cell surface staining as described in the previous section.

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.

b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).

3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 5 minutes at 400 RCF.
5. Discard supernatant and re-suspend in 1 mL of staining buffer + 0.1% permeabilizer.
6. Stain each sample at 1 uL/ 1 x 106 cells of primary antibody or 1-3 uL/ 1 x 106 cells for directly conjugated antibodies. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
7. Following the primary/conjugate incubation, add 2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 5 minutes at 400 RCF.
8. Remove supernatant and re-suspend each sample in 2 mL staining buffer + 0.1% permeabilizer, repeat wash for 5 minutes at 400 RCF.
9. If using a directly conjugated antibody, after the second wash, re-suspend cell pellet to a final volume of 500 uL per sample and proceed with flow analysis.

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Products Related to NBP1-49533

NB820-59465	MCF-7 Whole Cell Lysate
NBP1-49533PEP	Glut4 Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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