

Product Datasheet

Glut4 Antibody NBP1-49533SS

Unit Size: 0.025 ml

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

www.novusbio.com



support@novusbio.com

Reviews: 1 Publications: 1

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NBP1-49533

Updated 6/15/2014 v.20.1

NBP1-49533SS

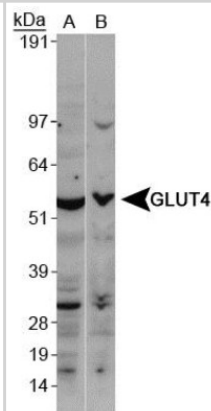
Glut4 Antibody

Product Information	
Unit Size	0.025 ml
Concentration	1.14 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS, 30% glycerol
Product Description	
Host	Rabbit
Gene ID	6517
Gene Symbol	SLC2A4
Species	Human, Mouse
Species Reactivity	Human and mouse. Predicted to react with rat based on 100% sequence homology.
Immunogen	A synthetic peptide made to a C-terminal portion of the human Glucose Transporter GLUT4 protein (between residues 480-509) [UniProt P14672]
Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Flow Cytometry, Immunocytochemistry/Immunofluorescence 1:100, Immunohistochemistry 1:200, Immunohistochemistry-Paraffin, Western Blot 0.5ug/ml
Application Notes	This GLUT4 antibody is useful for Western blot, IHC and ICC/IF. Use in Immunohistochemistry-Paraffin reported in scientific literature (PMID 24339864). Flow cytometry data from customer review.

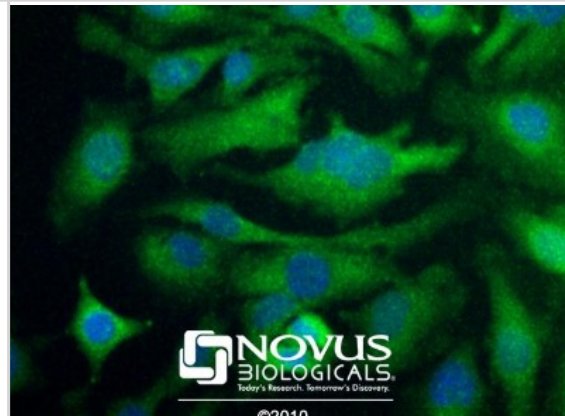


Images

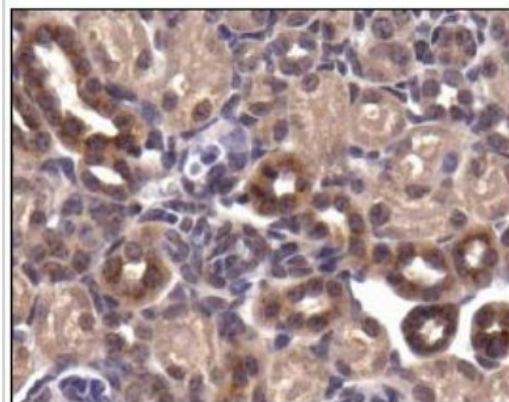
Western Blot: Glucose Transporter GLUT4 Antibody [NBP1-49533] - Analysis of GLUT4 in A. MCF7 whole cell lysate and B. 3T3L1 whole cell lysate



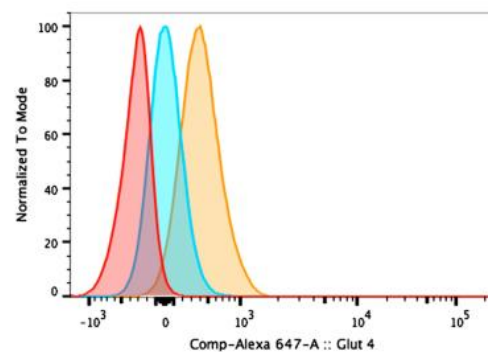
Immunocytochemistry/Immunofluorescence: Glucose Transporter GLUT4 Antibody [NBP1-49533] - Analysis of GLUT4 in HeLa cells



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



Flow Cytometry: Glut4 Antibody [NBP1-49533] - FLOW analysis 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. The Alexa Fluor (R) 647 conjugated version of this 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 submitted by Verified Customer - see Product Review on NBP1-49533AF647 for Protocol Details].



Publications

Gauger KJ, Bassa LM, Henchey EM et al. Mice Deficient in Sfrp1 Exhibit Increased Adiposity, Dysregulated Glucose Metabolism, and Enhanced Macrophage Infiltration. PLoS One 2013 Dec 5 [PMID: 24339864] (IHC-P, Mouse)



Procedures

Western Blot protocol specific for GLUT4 Antibody (NBP1-49533)

Western Blot Protocol

1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH₂O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% NFD₂M + 1% BSA in TBS + Tween, 1 hour at RT.
6. Rinse the membrane in dH₂O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-GLUT4 primary antibody (NBP1-49533) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH₂O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 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 manufacturers instructions (Pierce ECL).

****Note:** Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

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.

Staining:

1. Wash sections in dH₂O three times for 5 minutes each.
2. Wash section in wash buffer (1X PBS/0.1% Tween-20 (1X PBST)) for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1X PBST, 5% goat serum) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul primary antibody diluted in 1X PBST, 5% goat serum to each section. Incubate overnight at 4C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated secondary antibody, diluted in 1X PBST, 5% goat serum. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in dH₂O.
12. Counterstain sections in hematoxylin.
13. Wash sections in dH₂O two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Immunocytochemistry/Immunofluorescence Protocol for Glucose Transporter GLUT4 Antibody (NBP1-49533)

Immunocytochemistry Protocol

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

1. Pull off culture medium with and add 10% formalin to the dish. Fix at room temperature for 30 minutes..
2. Take off the formalin and add ice cold methanol (kept in well sealed bottle in -20C). Incubate for 5-10 minutes.
3. Take off methanol and add PBS (You can add 0.1% Tween-20 to PBS used here and all subsequent steps), be sure to not let the specimen dry out. Wash 3 times 10 minutes before proceeding to blocking step.
4. To block nonspecific antibody binding incubate in 10% normal goat serum for a minimum of 1 hr at room temp. Cells can also block overnight at 4C for this step.
5. Add primary antibody at appropriate dilution and incubate at room temp for 2 hrs or overnight at room temp.
6. Remove primary antibody and replace with PBS. Wash 3 x 10 min in PBS.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hr at room temperature
8. Remove antibody and replace with PBS, wash 1 x 10 min in PBS. Add Hoechst 33258 to PBS at 1:25,000 and incubate for 10 min. Wash a third time with PBS for 10 min (total of 3X10min PBS washes).
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide and parafilm. Cells can also be coverslipped using Fluoromount. If storing coverslip be sure to seal the edges with clear nail polish.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.





Novus Biologicals USA

8100 Southpark Way, A-8
Littleton, CO 80120
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
novus@novusbio.com

Novus Biologicals Canada

461 North Service Road West, Unit B37
Oakville, ON L6M 2V5
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada@novusbio.com

Novus Biologicals Europe

19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info@bio-techne.com

General Contact Information

www.novusbio.com
Technical Support: technical@novusbio.com
Orders: orders@novusbio.com
General: novus@novusbio.com

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.

For more information on our guarantee, please visit www.novusbio.com/guarantee.

