Product Datasheet

Glut4 Antibody
NBP1-49533

Unit Size: 0.1 ml
Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

Reviews: 2  Publications: 7

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Updated 1/23/2018 v.20.1

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NBP1-49533
Glut4 Antibody

**Product Information**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
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<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.02% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td>Buffer</td>
<td>PBS</td>
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**Product Description**

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<thead>
<tr>
<th>Feature</th>
<th>Details</th>
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</thead>
<tbody>
<tr>
<td>Host</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Gene ID</td>
<td>6517</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>SLC2A4</td>
</tr>
<tr>
<td>Species</td>
<td>Human, Mouse, Rat</td>
</tr>
<tr>
<td>Reactivity Notes</td>
<td>Human and mouse. Predicted to react with rat based on 100% sequence homology. Rat reactivity reported in scientific literature (PMID: 26468734)</td>
</tr>
<tr>
<td>Immunogen</td>
<td>A synthetic peptide made to a C-terminal portion of the human Glucose Transporter GLUT4 protein (between residues 480-509) [UniProt P14672]</td>
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**Product Application Details**

<table>
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<tr>
<th>Feature</th>
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<tr>
<td>Applications</td>
<td>Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin</td>
</tr>
<tr>
<td>Recommended Dilutions</td>
<td>Western Blot 0.5ug/ml, Flow Cytometry, Immunohistochemistry 1:200, Immunocytochemistry/Immunofluorescence 1:100, Immunohistochemistry-Paraffin, Flow (Intracellular)</td>
</tr>
<tr>
<td>Application Notes</td>
<td>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.</td>
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**Images**

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. This image was submitted via customer review.
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.

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-X100. 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 (Intracellular): Glut4 Antibody [NBP1-49533] - An intracellular stain was performed on HepG2 with Glut4 Antibody NBP1-49533 and a matched isotype control. Cells were fixed with 4% PFA and then permeabilized 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 (SA5-10033, Thermo Fisher).
Western Blot: Glut4 Antibody [NBP1-49533] - Analysis of GLUT4 in A. MCF7 whole cell lysate and B. 3T3L1 whole cell lysate


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 submitted by Verified Customer)
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### 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 µg 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% NFDM + 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 Striptavidin-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 paraflimed. 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.
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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