Product Datasheet

Glut1 Antibody - BSA Free
NB110-39113

Unit Size: 0.1 ml

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

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Reviews: 7 Publications: 62

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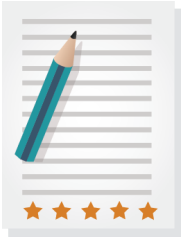
Updated 12/15/2024 v.20.1



NB110-39113

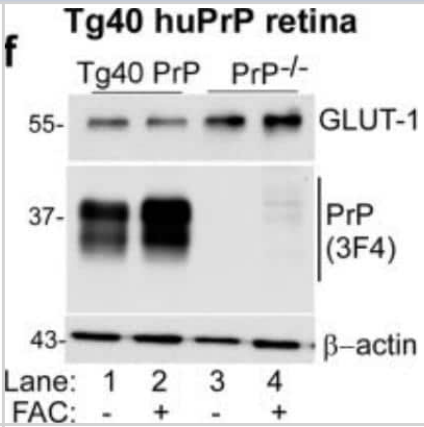
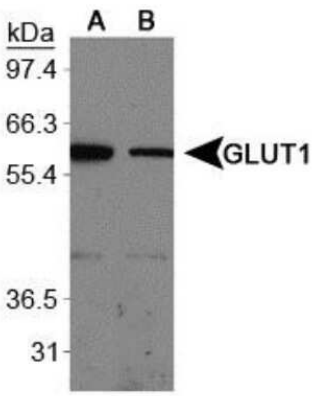
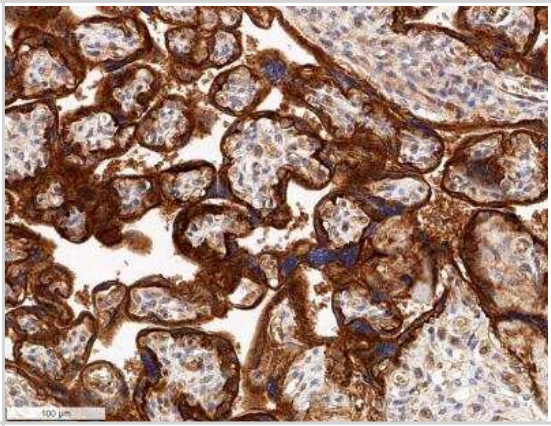
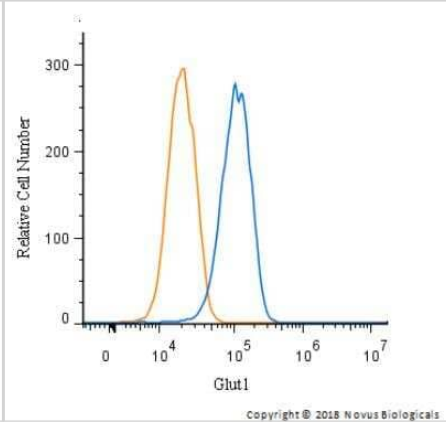
Glut1 Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1 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
Target Molecular Weight	54.1 kDa

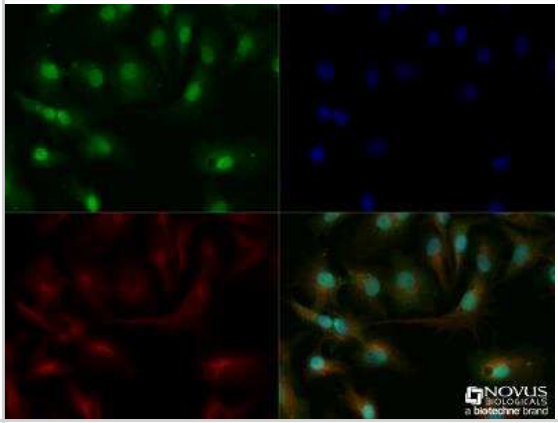
Product Description	
Host	Rabbit
Gene ID	6513
Gene Symbol	SLC2A1
Species	Human, Mouse, Rat, Rabbit
Reactivity Notes	Rabbit reactivity reported in scientific literature (PMID: 29456650). 100% sequence identity with primate, 93% sequence identity with bovine.
Marker	Plasma Membrane Marker
Immunogen	This Glut1 antibody is made against a synthetic peptide made to an N-terminal region of the human GLUT1 protein (between residues 1-100). [Swiss-Prot# P11166]. The immunogen is cytosolic.

Product Application Details	
Applications	Western Blot, Simple Western, Chromatin Immunoprecipitation, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vitro assay, Chromatin Immunoprecipitation (ChIP)
Recommended Dilutions	Western Blot 1:500, Simple Western, Chromatin Immunoprecipitation reported in scientific literature, Flow Cytometry 1 ug/ml. Use reported by customer review, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:1000, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen 1:200. Use reported in scientific literature, In vitro assay reported in scientific literature (Trachsel V et al), Flow (Intracellular) 1 ug/ml, Chromatin Immunoprecipitation (ChIP)
Application Notes	In WB a band is seen at ~55 kDa on kidney membrane preps representing GLUT1 protein. Depending on the tissue and any post-translational modifications, this protein can run anywhere between 40-60 kDa. See Simple Western Antibody Database for Simple Western validation: Tested in mouse eye tissue from D2 and D2G ON mouse model; separated by size; antibody dilution of 1:50.

Images

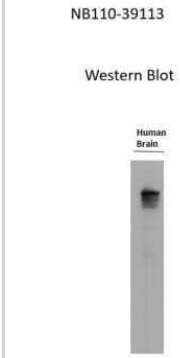
Western Blot: Glut1 Antibody [NB110-39113] - PrP-mediated increase in IC iron downregulates glucose transporters in the brain, neuroretina, and the liver: A similar evaluation of retinal lysates shows upregulation of Glut1 in PrP-/- relative to Tg40 PrP samples (lanes 1 & 3). Iron overloading downregulates Glut1 in Tg40 PrP samples relative to untreated controls, but has minimal effect on similarly treated PrP-/- samples (lanes 2 & 4). Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41598-018-24786-1) licensed under a CC-BY license.	
Western Blot: Glut1 Antibody [NB110-39113] - Western blot of GLUT1 on mouse kidney membrane protein (lane A) and rat kidney membrane protein (lane B).	
Immunohistochemistry-Paraffin: Glut1 Antibody [NB110-39113] - Immunohistochemical analysis of FFPE tissue section of human placenta using 1:200 dilution of Glut1 antibody. The staining was developed using HRP-DAB detection method and the sections were further counterstained with hematoxylin. This antibody generated a specific strong membrane cytoplasmic staining of Glut1 primarily in the syncytiotrophoblast layers of various villi and in the red blood cells (RBCs). Cytotrophoblasts showed a very weak expression of this protein.	
Flow Cytometry: Glut1 Antibody [NB110-39113] - An intracellular stain was performed on HepG2 with NB110-39113 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.	

Immunocytochemistry/Immunofluorescence: Glut1 Antibody [NB110-39113] - HepG2 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-GLUT1 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

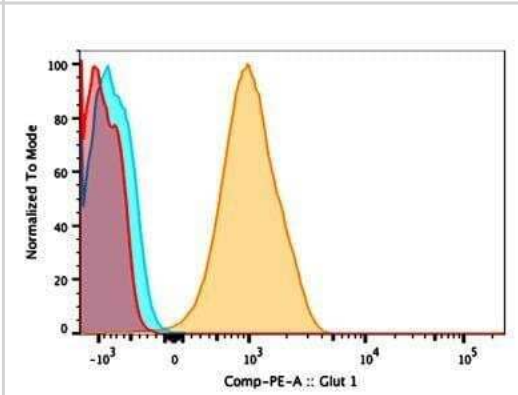


Western Blot: Glut1 Antibody [NB110-39113] - Analysis of HeLa lysates using NB110-39113. Image courtesy of Gregg Semenza (PMID: 21620138).

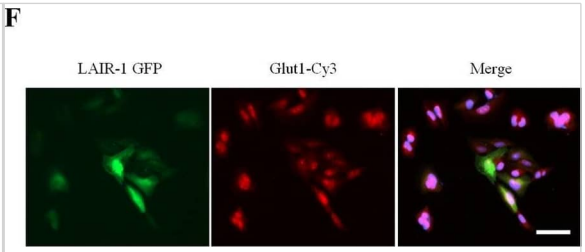
Western Blot: Glut1 Antibody [NB110-39113] - Glut1 in human brain lysate (55 kDa). Antibody at 1:500. Western blot image submitted by a verified customer review.



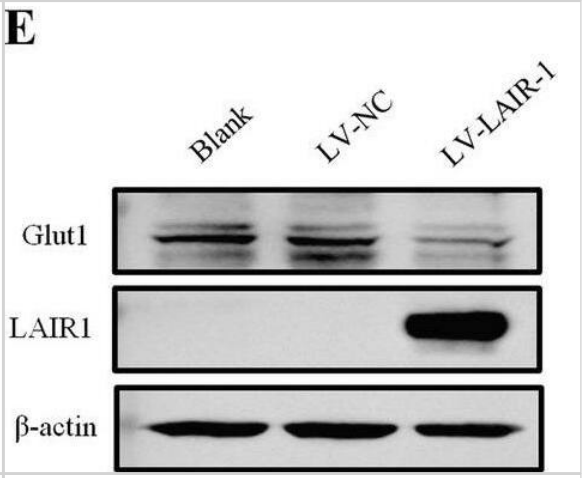
Flow Cytometry: Glut1 Antibody [NB110-39113] - Flow cytometry analysis using the PE conjugate of NB110-39113. Staining of Glut 1 expression on CD4+ T cells stimulated with anti-CD3/CD28 beads and insulin (1ug/mL) for 5 days in culture media with additional glucose provided. FMO control (red) and isotype control (blue, NBP2-24983) were compared to CD4+ T cells (orange), and this PE conjugated Glut 1 antibody positively stained CD4+ lymphocytes isolated from Mouse. Flow cytometry image submitted by a verified customer review.



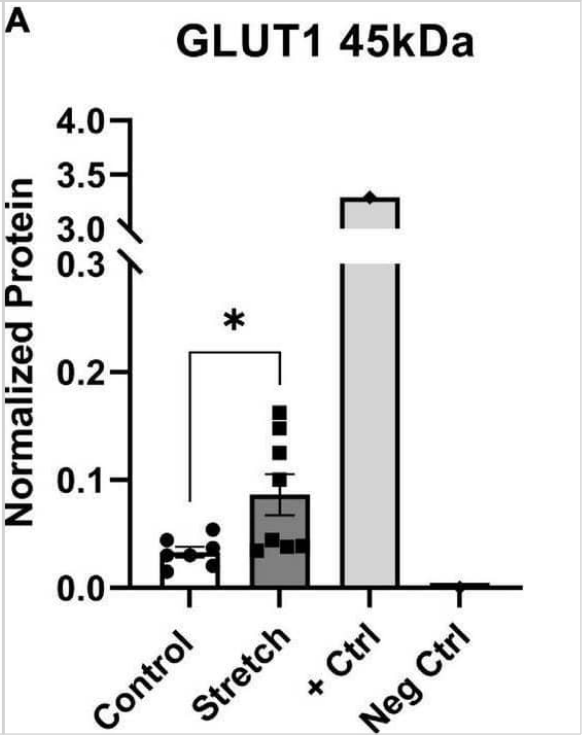
Immunocytochemistry/ Immunofluorescence: Glut1 Antibody - BSA Free [NB110-39113] - LAIR-1 inhibits Glut1-related glucose uptake in OS cells. a Heatmap showing the levels of differentially expressed mRNAs. b Top 20 KEGG pathway annotation categories for target gene functions of predicted mRNAs. c Selected significantly differentially expressed mRNA-related to EMT in RNA-seq data between two groups, ***P < 0.001. d qPCR validation of differentially expressed EMT-related genes in LV-NC & LV-LAIR-1-overexpressing OS cells, **P < 0.01. e Glut1 expression analyzed by western blotting. f Immunofluorescence staining of Glut1 in the LV-LAIR-1-overexpressing OS cells. Scale bar = 50 μm. Data were obtained from at least two independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32563267), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Glut1 Antibody - BSA Free [NB110-39113] - LAIR-1 inhibits Glut1-related glucose uptake in OS cells. a Heatmap showing the levels of differentially expressed mRNAs. b Top 20 KEGG pathway annotation categories for target gene functions of predicted mRNAs. c Selected significantly differentially expressed mRNA-related to EMT in RNA-seq data between two groups, ***P < 0.001. d qPCR validation of differentially expressed EMT-related genes in LV-NC & LV-LAIR-1-overexpressing OS cells, **P < 0.01. e Glut1 expression analyzed by western blotting. f Immunofluorescence staining of Glut1 in the LV-LAIR-1-overexpressing OS cells. Scale bar = 50 μm. Data were obtained from at least two independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32563267), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Simple Western: Glut1 Antibody - BSA Free [NB110-39113] - Protein changes in ONHAs corroborate bioenergetics data. (A) Glucose transporter-1 protein levels in Stretched ONH astrocytes are significantly higher than Control (*p = 0.0225, n = 7 Control, n = 8 Stretch). Retinal lysate from a 2 month-old mouse was used as a positive control for each protein analyzed, while negative control was the signal obtained when no primary antibody was included in the capillary. (B) Lactate dehydrogenase-A, the astrocyte-specific isoform of the enzyme that catalyzes the interconversion of pyruvate & lactate, has equivalent protein levels in Control & Stretch ONH astrocytes. (C) Glucose-6-phosphate dehydrogenase, the enzyme that shunts glucose into the pentose phosphate pathway, is no different in Control & Stretch ONH astrocytes. (D) Glutamine synthetase, the enzyme that synthesizes glutamine from glutamate, is no different in Control & Stretch ONH astrocytes. (E) The monomeric form of glutamate-aspartate transporter (GLAST) has significantly higher protein levels in the Stretch as compared to the Control ONH astrocytes (p = 0.020; n = 4 Control, n = 5 Stretch). (F) GLAST dimer protein levels are no different in Control & Stretch ONH astrocytes. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35992925), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Johnson RM, Olatunde AC, Woodie LN et al. The Systemic and Cellular Metabolic Phenotype of Infection and Immune Response to Listeria monocytogenes Frontiers in Immunology 2021-02-08 [PMID: 33628207]

Alexandria Béland-Millar, Alexia Kirby, Yen Truong, Julie Ouellette, Sozerko Yandiev, Khalil Bouyakdan, Chantal Pileggi, Shama Naz, Melissa Yin, Micaël Carrier, Pavel Kotchetkov, Marie-Kim St-Pierre, Marie-Ève Tremblay, Julien Courchet, Mary-Ellen Harper, Thierry Alquier, Claude Messier, Adam J Shuhendler, Baptiste Lacoste 16p11.2 haploinsufficiency reduces mitochondrial biogenesis in brain endothelial cells and alters brain metabolism in adult mice. Cell reports 2023-06-05 [PMID: 37149866]

Galiger C, Zohora FT, Dorneburg C et al. The survivin-ran inhibitor LLP-3 decreases oxidative phosphorylation, glycolysis and growth of neuroblastoma cells BMC cancer 2023-11-25 [PMID: 38007466]

Nishioku T, Anzai R, Hiramatsu S et al. Lactate dehydrogenase A inhibition prevents RANKL-induced osteoclastogenesis by reducing enhanced glycolysis Journal of Pharmacological Sciences 2023-12-01 [PMID: 37973217] (WB, Mouse)

Visavadiya NP, Rossiter HB, Khamoui AV. Distinct glycolytic pathway regulation in liver, tumour and skeletal muscle of mice with cancer cachexia Cell Biochemistry and Function 2021-08-01 [PMID: 34129243]

Bertelli PM, Pedrini E, Hughes D et al. Long term high glucose exposure induces premature senescence in retinal endothelial cells Frontiers in Physiology 2022-08-26 [PMID: 36091370]

He X, Zeng H, Cantrell AC et al. Knockout of TIGAR enhances myocardial phosphofructokinase activity and preserves diastolic function in heart failure Journal of Cellular Physiology 2022-08-01 [PMID: 35621078] (Western Blot, Block/Neutralize)

Nsiah NY, Inman DM. Destabilizing COXIV in M□ller Glia Increases Retinal Glycolysis and Alters Scotopic Electroretinogram Cells 2022-11-24 [PMID: 36497016] (Immunocytochemistry/ Immunofluorescence)

Jassim AH, Nsiah NY, Inman DM. Ocular Hypertension Results in Hypoxia within Glia and Neurons throughout the Visual Projection Antioxidants (Basel) 2022-04-29 [PMID: 35624752]

Artico LL, Ruas JS, Teixeira Júnior JR et al. IGFBP7 Fuels the Glycolytic Metabolism in B-Cell Precursor Acute Lymphoblastic Leukemia by Sustaining Activation of the IGF1R-Akt-GLUT1 Axis International journal of molecular sciences 2023-06-02 [PMID: 37298628] (FLOW)

García-Gómez P, Golán I, S Dadras M et al. NOX4 regulates TGF?-induced proliferation and self-renewal in glioblastoma stem cells Molecular oncology 2022-05-01 [PMID: 35203105] (WB, Human)

Details:
Multi-plex staining; Dilutions: 1:800

Wasser, Y, Y The Role of Dlk1 in the Gene Regulatory Network Underlying Motor Neuron Diversification Thesis 2022-01-01 (Western Blot, Mouse)

More publications at http://www.novusbio.com/NB110-39113

Procedures

Western Blot protocol for Glut1 Antibody (NB110-39113)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted 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 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 manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunocytochemistry/Immunofluorescence Protocol for Glut1 Antibody (NB110-39113)

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.

Immunohistochemistry-Paraffin Protocol for Glut1 Antibody (NB110-39113)
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 all the time).

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.

Flow (Intracellular) Protocol for Glut1 Antibody (NB110-39113)

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 100 uL 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 permeabilization 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:

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 permeabilization 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 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

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Products Related to NB110-39113

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NB110-39113C	Glut1 Antibody [DyLight 650]

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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