Product Datasheet

GPR81 Antibody - BSA Free
NLS2095

Unit Size: 0.05 ml

Keep as concentrated solution. Aliquot and store at -20C or below. Avoid multiple freeze-thaw cycles.

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Publications: 9

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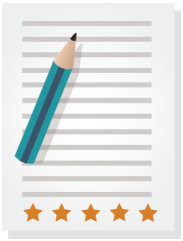
Updated 10/23/2024 v.20.1



NLS2095

GPR81 Antibody - BSA Free

Product Information

Unit Size	0.05 ml
Concentration	1.0 mg/ml
Storage	Keep as concentrated solution. Aliquot and store at -20C or below. Avoid multiple freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.1% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description

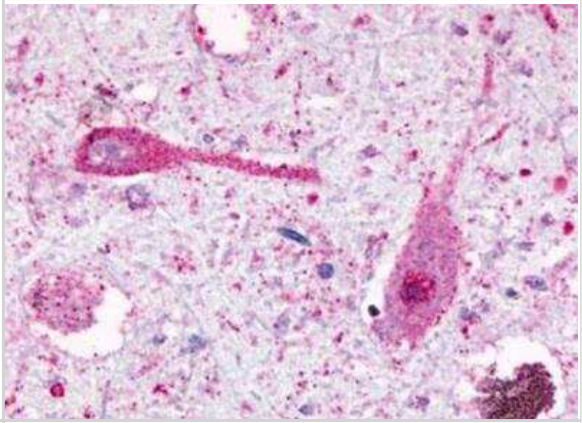
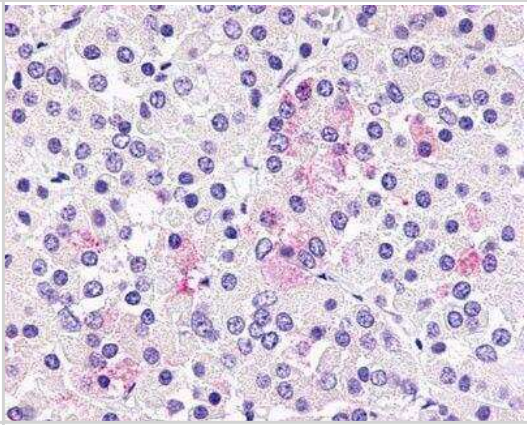
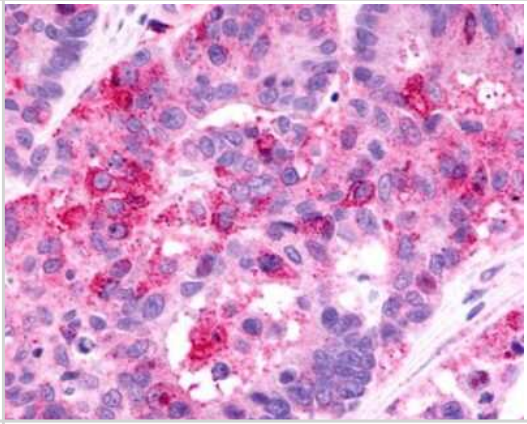
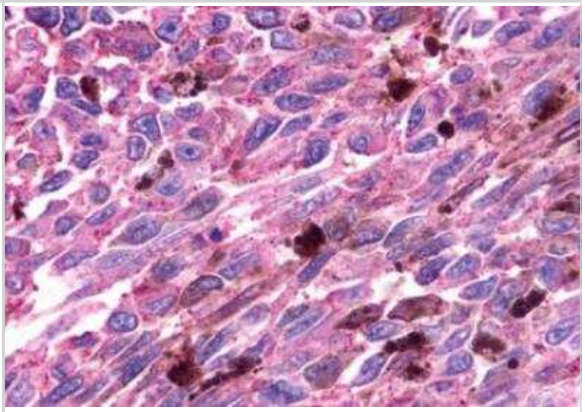
Description	Product can be stored undiluted at 4C for up to 1 month.
Host	Rabbit
Gene ID	27198
Gene Symbol	HCAR1
Species	Human, Mouse
Reactivity Notes	Predicted cross-reactivity based on sequence identity: Gorilla (100%), Marmoset (95%). Mouse reactivity reported in the literature (PMID: 19633298).
Specificity/Sensitivity	Human GPR81. BLAST analysis of the peptide immunogen showed no homology with other human proteins, except KRBA1 (53%).
Immunogen	Synthetic 19 amino acid peptide from C-terminus of human GPR81.

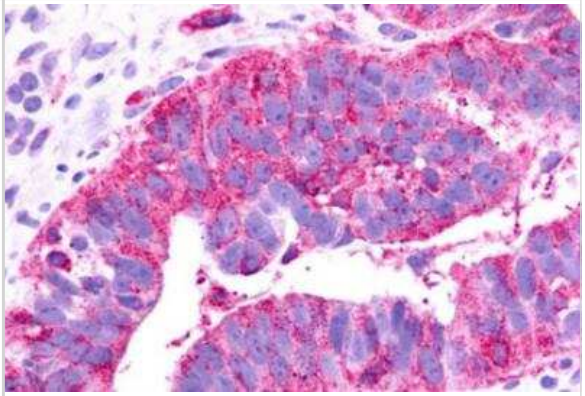
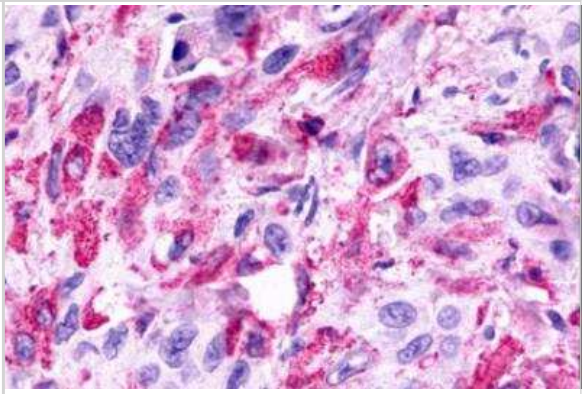
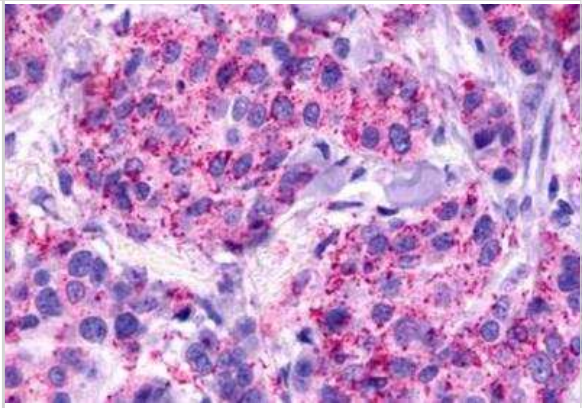
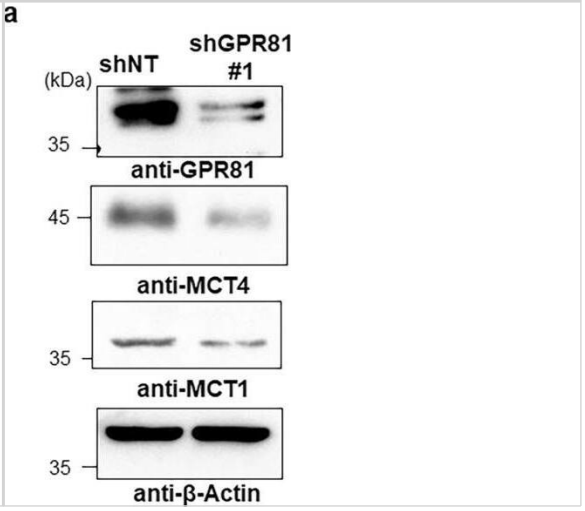
Product Application Details

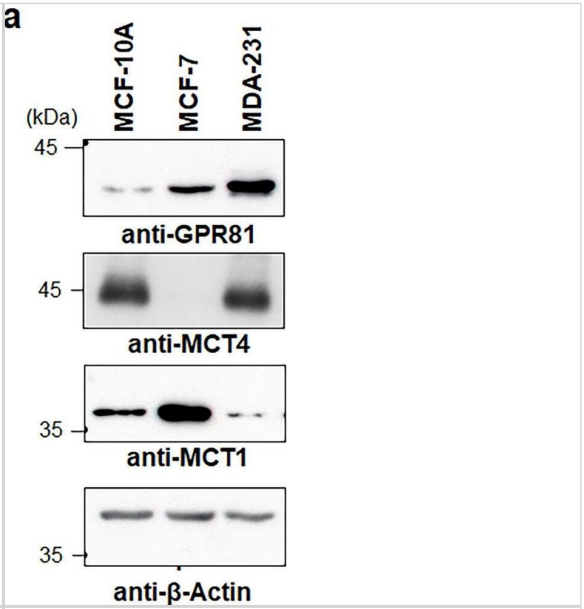
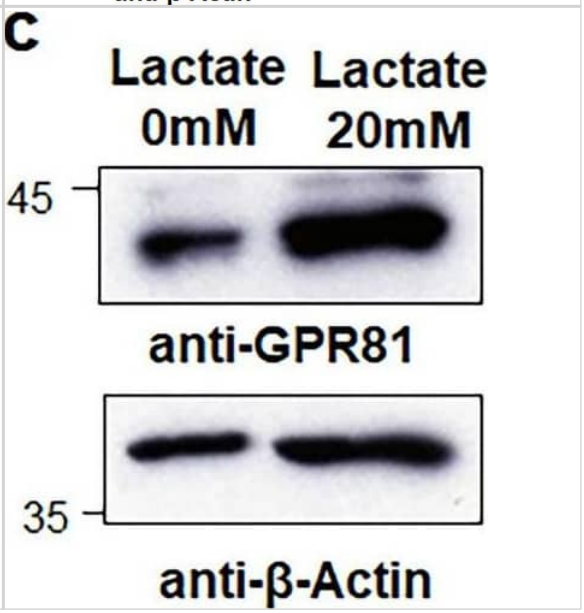
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot, Immunohistochemistry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry-Paraffin 2.5 - 20 ug/ml
Application Notes	WB reactivity reported in (PMID: 19633298). ICC/IF reactivity reported in (PMID: 32071396).

Images

Immunocytochemistry/Immunofluorescence: GPR81 Antibody [NLS2095] - LPS increases glycolysis as well as lactate production by BM neutrophils. MCT4, MCT1, and GPR81 (yellow) distribution on BM CD11b+ (green)/Ly6G+ (red) neutrophils visualized and quantified by ImageStream analysis. Images are from one representative experiment out of three. Scale bar indicates 7 um. Image collected and cropped by CiteAb from the following publication (www.nature.com/articles/s41467-020-17402-2) licensed under a CC-BY license.

Immunohistochemistry-Paraffin: GPR81 Antibody [NLS2095] - Brain, Substantia Nigra, pigmented and nonpigmented neurons.	
Immunohistochemistry-Paraffin: GPR81 Antibody [NLS2095] - Analysis of anti-FKSG80 / GPR81 antibody with human anterior pituitary.	
Immunohistochemistry-Paraffin: GPR81 Antibody [NLS2095] - Analysis of anti-FKSG80 / GPR81 antibody with human colon, carcinoma.	
Immunohistochemistry-Paraffin: GPR81 Antibody [NLS2095] - Human Skin, Melanoma formalin-fixed, paraffin-embedded tissue after heat-induced antigen retrieval.	

Immunohistochemistry-Paraffin: GPR81 Antibody [NLS2095] - Human Ovary, Carcinoma formalin-fixed, paraffin-embedded tissue after heat-induced antigen retrieval.	
Immunohistochemistry-Paraffin: GPR81 Antibody [NLS2095] - Human pancreas, carcinoma formalin-fixed, paraffin-embedded tissue after heat-induced antigen retrieval.	
Immunohistochemistry-Paraffin: GPR81 Antibody [NLS2095] - Breast, Carcinoma	
Western Blot: GPR81 Antibody - BSA Free [NLS2095] - Stable knockdown of GPR81 in MDA-MB-231 cells. Using a lentiviral system, GPR81 was stably knocked down in MDA-MB-231 cells with GPR81-specific short hairpin RNA (shGPR81 #1) & compared with cells containing scrambled shRNA as a control (shNT). Knockdown of GPR81 was confirmed by (a) western blotting & (b) RT-qPCR. The GPR81, MCT1, & MCT4 mRNA levels in the shGPR81 #1 cells are presented as the fold increase compared with those in shNT cells. Data are presented as the means ± s.d. (n = 3/group). **p < 0.01; Student's t-test. (c) Lactate production in shNT & shGPR81 #1 cells. The cells were cultured for 24 h & lactate concentrations in the supernatants were determined (n = 3/group). **p < 0.01 (vs. shNT cells); Student's t-test. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35428832), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Western Blot: GPR81 Antibody - BSA Free [NLS2095] - Expression of GPR81 in breast cancer cells. (a) Western blot analysis of GPR81 in breast cancer cells (MCF-7 & MDA-MB-231) & non-tumorigenic epithelial cells (MCF-10A). Cell lysates were analyzed by immunoblotting using anti-GPR81, anti-MCT4, anti-MCT1, & anti-β-actin antibodies. (b) Immunocytochemical analysis of GPR81 expression in breast cancer cells (MCF-7 & MDA-MB-231) & non-tumorigenic epithelial cells (MCF-10A). MCF-10A, MCF-7 & MDA-MB-231 cells were incubated with polyclonal antibodies against GPR81 followed by Alexa Fluor Plus 488-conjugated secondary antibody & visualized under a fluorescence microscope. Na/K ATPase was used as a cell membrane marker & the nuclei were stained with DAPI. Scale bar 10 µm. (c) MDA-MB-231 cells were cultured in the presence & absence of 20 mM lactate for 48 h, & cell lysates were analyzed by immunoblotting using anti-GPR81 & anti-β-actin antibodies. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35428832), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: GPR81 Antibody - BSA Free [NLS2095] - Expression of GPR81 in breast cancer cells. (a) Western blot analysis of GPR81 in breast cancer cells (MCF-7 & MDA-MB-231) & non-tumorigenic epithelial cells (MCF-10A). Cell lysates were analyzed by immunoblotting using anti-GPR81, anti-MCT4, anti-MCT1, & anti-β-actin antibodies. (b) Immunocytochemical analysis of GPR81 expression in breast cancer cells (MCF-7 & MDA-MB-231) & non-tumorigenic epithelial cells (MCF-10A). MCF-10A, MCF-7 & MDA-MB-231 cells were incubated with polyclonal antibodies against GPR81 followed by Alexa Fluor Plus 488-conjugated secondary antibody & visualized under a fluorescence microscope. Na/K ATPase was used as a cell membrane marker & the nuclei were stained with DAPI. Scale bar 10 µm. (c) MDA-MB-231 cells were cultured in the presence & absence of 20 mM lactate for 48 h, & cell lysates were analyzed by immunoblotting using anti-GPR81 & anti-β-actin antibodies. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35428832), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Ruishuang Ma, Xin Li, Shengping Gong, Xiaoqin Ge, Ting Zhu, Xiaoxu Ge et al. Dual Roles of Lactate in EGFR-TKI-Resistant Lung Cancer by Targeting GPR81 and MCT1 Journal of Oncology 2022-12-12 [PMID: 36545125]

Liu W, Zhang S, Li Q et al. Lactate modulates iron metabolism by binding soluble adenylyl cyclase Cell metabolism 2023-07-18 [PMID: 37480842]

Zhang L, Wang S, Ma Y et al. Shoutai Wan regulates glycolysis imbalance at the maternal-fetal interface in threatened abortion mice Journal of ethnopharmacology 2023-04-15 [PMID: 37068718] (WB, IHC-P, Mouse)

Details:
IHC-P Dilution: 1:100; WB Dilution: 1:200

Okui T, Hiasa M, Hasegawa K et al. Lactate secreted via MCT4 from bone?colonizing breast cancer excites sensory neurons via GPR81 International journal of oncology 2023-03-01 [PMID: 36799150] (WB, Rat)

Details:
Dilution used in WB 1:00

Ishihara S, Hata K, Hirose K et al. The lactate sensor GPR81 regulates glycolysis and tumor growth of breast cancer Scientific reports 2022-04-15 [PMID: 35428832] (WB, Mouse)

Murakami R, Chiba Y, Nishi N et al. Immunoreactivity of receptor and transporters for lactate located in astrocytes and epithelial cells of choroid plexus of human brain Neurosci Lett 2020-11-16 [PMID: 33212210] (ICC/IF, IF/IHC, Human)

Details:
Immunofluorescence analysis and immunohistochemical analysis of human brain samples. Western blot analysis performed in HeLa and HT-29.

Brown TP, Bhattacharjee P, Ramachandran S et al. The lactate receptor GPR81 promotes breast cancer growth via a paracrine mechanism involving antigen-presenting cells in the tumor microenvironment Oncogene 2020-02-19 [PMID: 32071396] (ICC/IF, Mouse)

de Castro Abrantes H, Briquet M, Schmuziger C, et al. The Lactate Receptor HCAR1 Modulates Neuronal Network Activity through the Activation of G alpha and G beta gamma Subunits J. Neurosci. 2019-06-05 [PMID: 30926749] (WB, Mouse)

Jeninga EH, Bugge A, Nielsen R et al. Peroxisome proliferator-activated receptor gamma regulates expression of the anti-lipolytic G-protein-coupled receptor 81 (GPR81/Gpr81). J Biol Chem 2009-09-25 [PMID: 19633298] (WB, Mouse)

Procedures

Immunohistochemistry protocol for GPR81 Antibody (NLS2095)
Immunohistochemistry Protocol for GPR81 Antibody (NLS2095):
Immunohistochemistry

1. Prepare tissue with formalin fixation and by embedding it in paraffin wax.
2. Make 4 um sections and place on pre-cleaned and charged microscope slides.
3. Heat in a tissue-drying oven for 45 minutes @ 60 degrees Celcius.
4. Deparaffinize the tissues by wash drying the slides in 3 changes of xylene for 5 minutes each @ RT.
5. Rehydrate the tissues by washing the slides in 3 changes of 100% alcohol for 3 minutes each @ RT.
6. Wash the slides in 2 changes of 95% alcohol for 3 minutes each @ RT.
7. Wash the slides in 1 change of 80% alcohol for 3 minutes @ RT.
8. Rinse the slides in gentle running distilled water for 5 minutes @ RT.
9. Perform antigen retrieval by steaming the slides in 0.01M sodium citrate buffer (pH 6.0) @ 99-100 degrees Celcius for 20 minutes.
10. Remove the slides from the heat and let stand in buffer @ RT for 20 minutes.
11. Rinse the slides in 1X TBS-T for 1 minute @ RT.

Do not allow the tissues to dry at any time during the staining procedure

12. Begin the immunostaining by applying a universal protein block for 20 minutes @ RT.
13. Drain protein block from the slides and apply the diluted primary antibody for 45 minutes @ RT.
14. Rinse the slide in 1X TBS-T for 1 minute @ RT.
15. Apply a biotinylated anti-rabbit IgG (H+L) secondary for 30 minutes @ RT.
16. Rinse the slide in 1X TBS-T for 1 minute @ RT.
17. Apply an alkaline phosphatase steptavidin for 30 minutes @ RT.
18. Rinse the slide in 1X TBS-T for 1 minute @ RT.
19. Apply an alkaline phosphatase chromagen substrate for 30 minutes @ RT.
20. Rinse the slide in distilled water for 1 minute @ RT.

This method should only be used if the chromagen substrate is alcohol insoluble (ie: Vector Red, DAB)

21. Dehydrate the tissue by washing the slides in 2 changes of 80% alcohol for 1 minute each @ RT.
22. Wash the slides in 2 changes of 95% alcohol for 1 minute each @ RT.
23. Wash the slides in 3 changes of 100% alcohol for 1 minute each @ RT.
24. Wash the slides in 3 changes of xylene for 1 minute each @ RT.
25. Apply cover slip.

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Products Related to NLS2095

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
M6000B-1	IL-6 [HRP]

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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