Product Datasheet

Lgr5/GPR49 Antibody - BSA Free
NBP1-28904

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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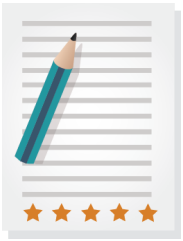
Updated 5/21/2024 v.20.1



NBP1-28904

Lgr5/GPR49 Antibody - BSA Free

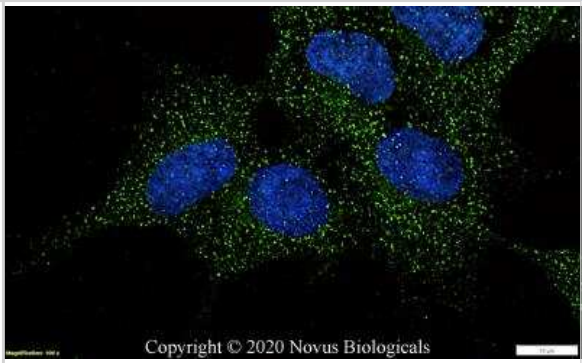
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	8549
Gene Symbol	LGR5
Species	Human, Mouse, Porcine
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID:32016468).
Immunogen	Synthetic peptide made to an internal portion of the human GPR49/LGR5 protein (within residues 650-700). [Swiss-Prot: O75473]

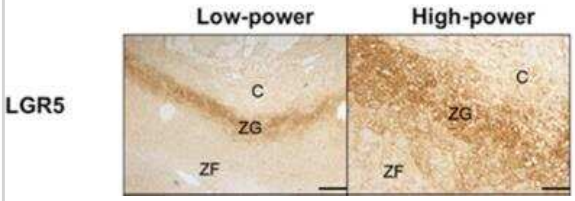
Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1.0-2.0 ug/ml, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 2-5 ug/ml, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen

Images

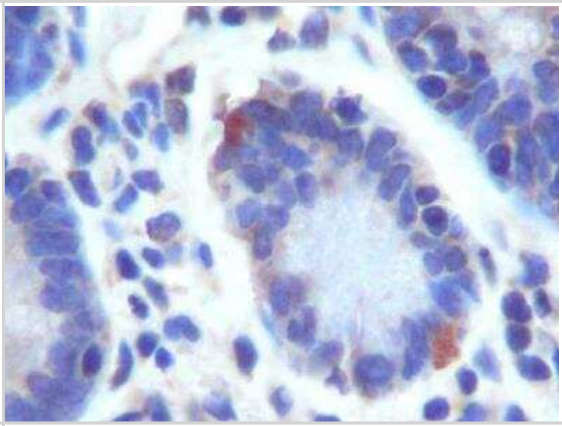
Immunocytochemistry/Immunofluorescence: Lgr5/GPR49 Antibody [NBP1-28904] - Hek293 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-Lgr5/GPR49 Antibody NBP1-28904 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



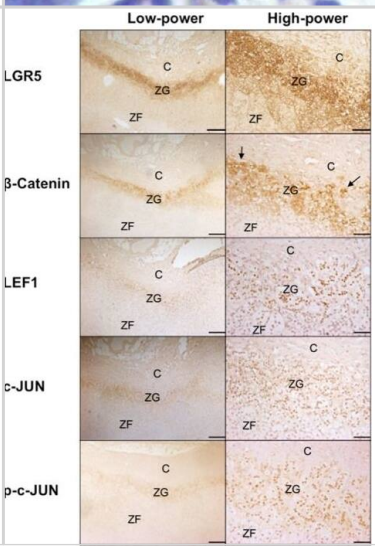
Immunohistochemistry: Lgr5/GPR49 Antibody [NBP1-28904] - Wnt-related genes show selective expression in the ZG of human adrenal. IHC localization of LGR5 in the ZG. IHC was performed on formalin-fixed, paraffin-embedded human adrenal sections (4 um) using a chromogen-based detection system (3,3'-Diaminobenzidine), which results in a positive brown staining. Pictures are representative of six normal adrenal sections that were used in our microarray study; four from primary hyperaldosteronism patients and two from pheochromocytoma patients. Scale bar, 500 um (low power) and 100 um (high power). C, capsule. Image collected and cropped by CiteAb from the following publication (https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2015-1734), licensed under a CC-BY license.



Immunohistochemistry: Lgr5/GPR49 Antibody [NBP1-28904] - Analysis of human small intestine.



Wnt-related genes show selective expression in the ZG of human adrenal. B) IHC localization of LGR5 and downstream Wnt signaling proteins in the ZG: from the canonical beta-catenin pathway, beta-catenin and LEF1, and from the noncanonical AP1/JUN pathway, c-JUN and p-c-JUN. IHC was performed on formalin-fixed, paraffin-embedded human adrenal sections (4 um) using a chromogen-based detection system (3,3'-Diaminobenzidine), which results in a positive brown staining. Interestingly, beta-catenin staining was mostly membranous or cytoplasmic (rather than nuclear staining as highlighted by the arrows), indicating limited canonical Wnt activation. Pictures are representative of six normal adrenal sections that were used in our microarray study; four from primary hyperaldosteronism patients and two from pheochromocytoma patients. Scale bar, 500 um (low power) and 100 um (high power). C, capsule. Image collected and cropped by CiteAb from the following publication (https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2015-1734), licensed under a CC-BY licence.



Publications

Xie Y, Ding F, Di W et al. Impact of a high□fat diet on intestinal stem cells and epithelial barrier function in middle□aged female mice Mol Med Rep 2020-02-05 [PMID: 32016468] (IHC-P, IHC-P, Mouse)

Zhang R, Zhang X, Zhang W et al. Sohlh2 regulates the stemness and differentiation of colon cancer stem cells by downregulating LncRNA-H19 transcription Molecular cancer research : MCR 2022-10-26 [PMID: 36287177] (WB, Human)

Zhang R, Liu L, Wang F et al. AKAP8L enhances the stemness and chemoresistance of gastric cancer cells by stabilizing SCD1 mRNA Research Square 2022-08-25 [PMID: 36522343] (WB, Human)

Di W, Lv Y, Xia F et al. Improvement of intestinal stem cells and barrier function via calorie restriction in middle-aged C57BL/6 mice Nutr Res 2020-09-03 [PMID: 32877836]

Lee J, Kim JS, Cho HI et al. JIB-04, a Pan-Inhibitor of Histone Demethylases, Targets Histone-Lysine-Demethylase-Dependent AKT Pathway, Leading to Cell Cycle Arrest and Inhibition of Cancer Stem-Like Cell Properties in Hepatocellular Carcinoma Cells International journal of molecular sciences 2022-07-11 [PMID: 35887001] (WB, Human)

Akbari S, Kunter I, Azbazdar Y et al. LGR5/R-Spo1/Wnt3a axis promotes stemness and aggressive phenotype in hepatoblast-like hepatocellular carcinoma cell lines Cellular signalling 2021-03-06 [PMID: 33684507] (WB)

von Erlach T, Saxton S, Shi Y et al. Robotically handled whole-tissue culture system for the screening of oral drug formulations Nat Biomed Eng 2020-04-27 [PMID: 32341538]

Kim MS, Cho HI, Yoon HJ et al. JIB-04, A Small Molecule Histone Demethylase Inhibitor, Selectively Targets Colorectal Cancer Stem Cells by Inhibiting the Wnt/B-Catenin Signaling Pathway Sci Rep 2018-04-26 [PMID: 29700375] (WB, Human)

Cervello I, Gil-Sanchis C, Santamaria X et al. Leucine-rich repeat-containing G-protein-coupled receptor 5-positive cells in the endometrial stem cell niche. Fertil. Steril. 2016-11-22 [PMID: 27887719]

Shaikh LH, Zhou J, Teo AE et al. LGR5 activates non-canonical Wnt-signaling and inhibits aldosterone production in the human adrenal J. Clin. Endocrinol. Metab. 2015-04-27 [PMID: 25915569] (IHC-P, Human)

Details:
Lgr5/GPR49 antibody used for Immunohistochemistry on formalin-fixed, paraffin-embedded human adrenal sections.

Jung K, Saif LJ. Porcine epidemic diarrhea virus infection: Etiology, epidemiology, pathogenesis and immunoprophylaxis. Vet J. 2015-02-26 [PMID: 25841898] (IHC-Fr, Porcine)

Details:
GPR49/LGR5 antibody was used in IHC-Fr application for studying the immunolocalization of LGR5+ crypt stem cells in the small intestine of gnotobiotic pigs inoculated with US porcine epidemic diarrhea virus/PEDV strain PC21A. Fig 4 depicts the LGR5+ crypt stem cells and LGR5+ positive crypt base columnar cells in the Jejunum section of a PEDV-inoculated pig at post-inoculation hour 30, and this data confirms the presence of stem cells which are highly important as far as the epithelial cell renewal during acute-stage of PEDV infection is concerned.

Procedures

Immunohistochemistry-Paraffin protocol for Lgr5/GPR49 Antibody (NBP1-28904)
Lgr5/GPR49 Antibody:
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.

Immunostaining:
1. After the antigen retrieval, wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution 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 biotinylated diluted secondary antibody. 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 deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Western Blot Protocol for Lgr5/GPR49 Antibody (NBP1-28904)
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.

Immunocytochemistry/ Immunofluorescence Protocol for Lgr5/GPR49 Antibody (NBP1-28904)
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.

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Products Related to NBP1-28904

NBP1-28904PEP	Lgr5/GPR49 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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