Product Datasheet

TGR5/GPBAR1 Antibody - BSA Free NBP2-23669

Unit Size: 0.1 ml

Store at -20C. Avoid freeze-thaw cycles.

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Updated 10/23/2024 v.20.1

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NBP2-23669

TGR5/GPBAR1 Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at -20C. 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	151306
Gene Symbol	GPBAR1
Species	Human, Mouse, Rat
Reactivity Notes	Bovine (86%).
Immunogen	Synthetic peptide from C-terminus of the human TGR5/GPBAR1 protein [UniProt Q8TDU6]
Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Knockdown Validated
Recommended Dilutions	Western Blot 1 - 2 ug/ml, Immunohistochemistry 1:200-1:500, Immunocytochemistry/ Immunofluorescence 2 ug/ml, Immunohistochemistry- Paraffin 1:200-1:500, Knockdown Validated

Images

Western Blot: TGR5/GPBAR1 Antibody [NBP2-23669] - Analysis of spleen lysate. (1) Pre-immune sera. TGR5/GPBAR1 antibody tested on (2) Human 1:1000, (3) Mouse 1:5000, and (4) Rat 1:5000 spleen lysates.





Immunocytochemistry/Immunofluorescence: TGR5/GPBAR1 Antibody [NBP2-23669] - 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-TGR5/GPBAR1 Antibody NBP2-23669 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-Paraffin: TGR5/GPBAR1 Antibody [NBP2-23669] - Tissue section of mouse colon using TGR5/GPBAR1 antibody at 1:200 dilution. The signal was detected using HRP-labelled secondary antibody and DAB staining which followed counterstaining with hematoxylin. This TGR5/GPBAR1 antibody generated a very specific a cytoplasmic staining primarily in the columner epithelial cells. A subset of cells from mucosa muscularis and the lamina propria also showed TGR5 positivity. Some cells in all histological layers depicted a nuclear staining also.

Immunocytochemistry/Immunofluorescence: TGR5/GPBAR1 Antibody [NBP2-23669] - Hek293 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton X-100. The cells were incubated with anti-TGR5/GPBAR1 at 2 ug/mL 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.

Knockdown Validated: TGR5/GPBAR1 Antibody [NBP2-23669] - LCAinduced oxidative stress responses were mediated by TGR5/GPBAR1 and by CAR receptors. TGR5/GPBAR1, CAR, VDR, and PXR bile acid receptors were silenced in MCF7 cells by transiently transfecting the cells with the corresponding siRNA or a negative control siRNA. After 48 h, protein expressions of NRF2 and iNOS were determined by western blotting (n = 3). Data are plotted as mean +/- SEM. * p < 0.05, control vs. LCA/siRNA treated. (CAR, constitutive androstane receptor; FXR, farnesoid X-activated receptor; LCA, lithocholic acid; LXR, liver X nuclear receptor; NRF2, nuclear factor, erythroid 2-like 2; TGR5/GPBAR1, G protein-coupled bile acid receptor 1/Takeda G-protein coupled receptor; VDR, vitamin D receptor). Image collected and cropped by CiteAb from the following publication (https://www.mdpi.com/2072-6694/11/9/1255), licensed under a CC-BY license.



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С Western Blot: TGR5/GPBAR1 Antibody - BSA Free [NBP2-23669] -LCA-induced oxidative stress responses were mediated by TGR5 & by CAR receptors. (A) The 4T1 cells were treated with 0.3 µm LCA & NF449, CINPA1, DY268, or GSK2033 at a final concentration of 5 µm for 48 h, then NRF2 protein expression was detected using western blotting (representative figure, n = 2). (B,C) TGR5, CAR, VDR, & PXR bile acid receptors were silenced in MCF7 cells by transiently transfecting the cells with the corresponding siRNA or a negative control siRNA. After 48 h, protein expressions of (B,C) NRF2 & (C) iNOS were determined by western blotting (n = 3). Data are plotted as mean \pm SEM. * p < 0.05, control vs. LCA/siRNA treated. (CAR, constitutive androstane receptor; FXR, farnesoid X-activated receptor; LCA, lithocholic acid; LXR, liver X nuclear receptor; NRF2, nuclear factor, erythroid 2-like 2; TGR5/GPBAR1, G protein-coupled bile acid receptor 1/Takeda G-protein coupled receptor; VDR, vitamin D receptor). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31461945), licensed under a CC-BY

license. Not internally tested by Novus Biologicals.





Publications

Zhong S, Liu F, Giniatullin R et al. Blockade of CCR5 suppresses paclitaxel-induced peripheral neuropathic pain caused by increased deoxycholic acid Cell reports 2023-11-28 [PMID: 37948181] (WB, IHC, Rat)

Details:

WB dilution 1:1000; IHC dilution 1:200

Biagioli M, Marchianò S, Di Giorgio C et al. Activation of GPBAR1 attenuates vascular inflammation and atherosclerosis in a mouse model of NAFLD-related cardiovascular disease Biochemical Pharmacology 2023-11-01 [PMID: 37926268] (IHC-P, ICC/IF, Human, Mouse)

Zhao W, Chen L, Tan W et al. Mannan Oligosaccharides Promoted Skeletal Muscle Hypertrophy through the Gut Microbiome and Microbial Metabolites in Mice Foods 2023-01-12 [PMID: 36673449] (Western Blot)

Zhai Z, Niu KM, Liu Y et al. The Gut Microbiota-Bile Acids-TGR5 Axis Mediates Eucommia ulmoides Leaf Extract Alleviation of Injury to Colonic Epithelium Integrity Frontiers in Microbiology 2021-08-18 [PMID: 34489916]

Xu J, Li X, Yao X et al. Protective Effects of Bile Acids Against Hepatic Lipid Accumulation in Hybrid Grouper Fed a High-Lipid Diet Frontiers in Nutrition 2022-01-25 [PMID: 35145986] (Western Blot)

Di Giorgio C, Bellini R, Lupia A et al. Discovery of BAR502, as potent steroidal antagonist of leukemia inhibitory factor receptor for the treatment of pancreatic adenocarcinoma Frontiers in oncology 2023-03-14 [PMID: 36998446] (ICC/IF, Human)

Han B, Lv X, Liu G et al. Gut microbiota-related bile acid metabolism-FXR/TGR5 axis impacts the response to anti-? 4?7-integrin therapy in humanized mice with colitis Gut microbes 2023-07-11 [PMID: 37431863] (WB)

Zou Y, Ghaderpour A, Munkhbileg B et al. Taurodeoxycholate ameliorates DSS-induced colitis in mice International immunopharmacology 2023-07-14 [PMID: 37454634] (Immunocytochemistry/ Immunofluorescence)

Meng Y, Li W, Hu C et al. Ginsenoside F1 administration promotes UCP1-dependent fat browning and ameliorates obesity-associated insulin resistance Food Science and Human Wellness 2023-11-01

Tawulie D, Jin L, Shang X et al. Jiang-Tang-San-Huang pill alleviates type 2 diabetes mellitus through modulating the gut microbiota and bile acids metabolism Phytomedicine : international journal of phytotherapy and phytopharmacology 2023-02-26 [PMID: 36870307]

Hashmi SFUH THE MECHANISTIC AND ETIOLOGICAL LINK BETWEEN BILE ACID DYSREGULATION AND PRETERM BIRTH Thesis 2022-01-01 (WB, Mouse)

Wang LJ, Zhao GP, Wang XF et al. Glycochenodeoxycholate Affects Iron Homeostasis via Up-Regulating Hepcidin Expression Nutrients 2022-08-02 [PMID: 35956351] (WB, Mouse, Human)

Details: Dilution used 1:1000

More publications at http://www.novusbio.com/NBP2-23669



Procedures

Western Blot protocol for TGR5/GPBAR1 Antibody (NBP2-23669)

TGR5/GPBAR1 Antibody: 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 manufacturers instructions.

Immunocytochemistry/Immunofluorescence protocol for TGR5/GPBAR1 Antibody (NBP2-23669)

TGR5/GPBAR1 Antibody: 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 TGR5/GPBAR1 Antibody (NBP2-23669)

TGR5/GPBAR1 Antibody:

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 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.





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Products Related to NBP2-23669

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NBP2-23669PEP	TGR5/GPBAR1 Antibody Blocking Peptide

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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