Product Datasheet

15-PGDH/HPGD Antibody
NB200-179

Unit Size: 0.1 ml

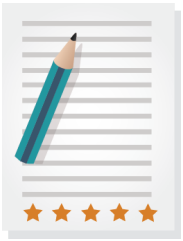
Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

www.novusbio.com technical@novusbio.com

Reviews: 3 Publications: 29

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB200-179

Updated 12/20/2023 v.20.1



NB200-179

15-PGDH/HPGD Antibody

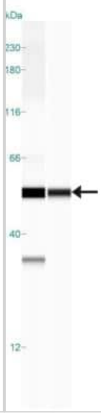
Product Information	
Unit Size	0.1 ml
Concentration	2 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Protein A purified
Buffer	PBS (pH 7.4)

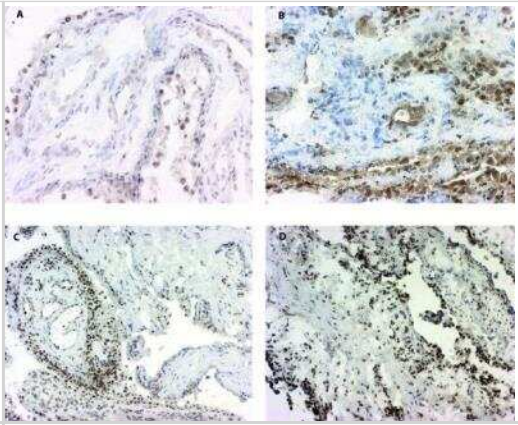
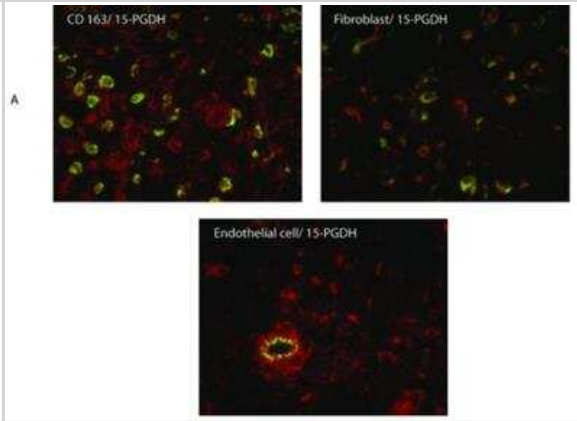
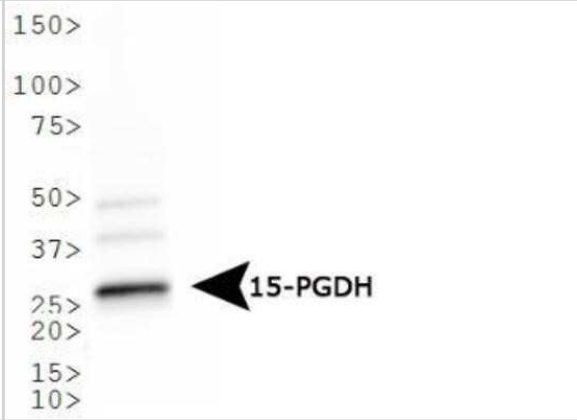
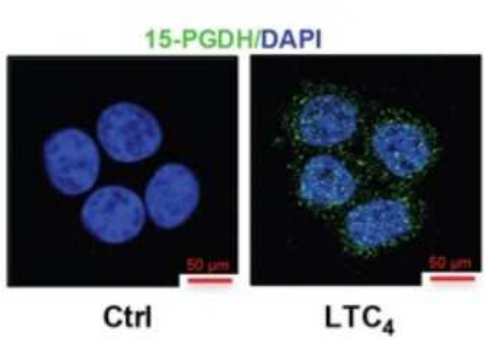
Product Description	
Host	Rabbit
Gene ID	3248
Gene Symbol	HPGD
Species	Human, Mouse, Rat
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 27140190) Human reactivity reported in scientific literature (PMID:33210098).
Immunogen	Purified type I human placental 15-PGDH protein [UniProt# P15428]

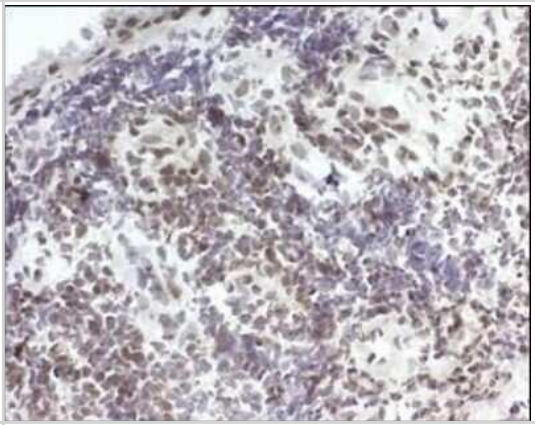
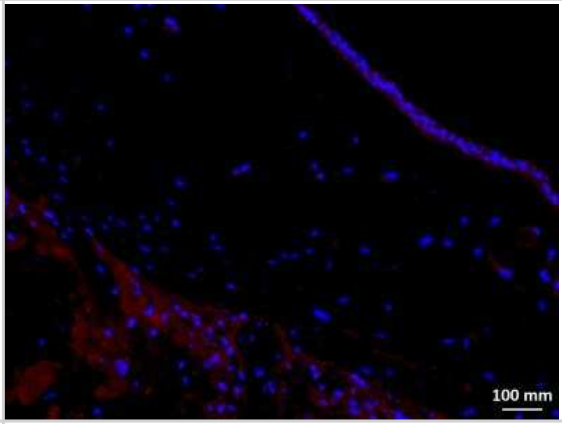
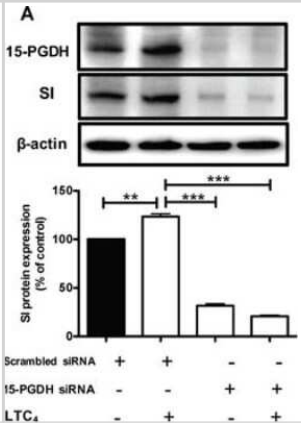
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Knockdown Validated
Recommended Dilutions	Western Blot 1:5000-1:6000, Simple Western 1:500, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen, Knockdown Validated

Images

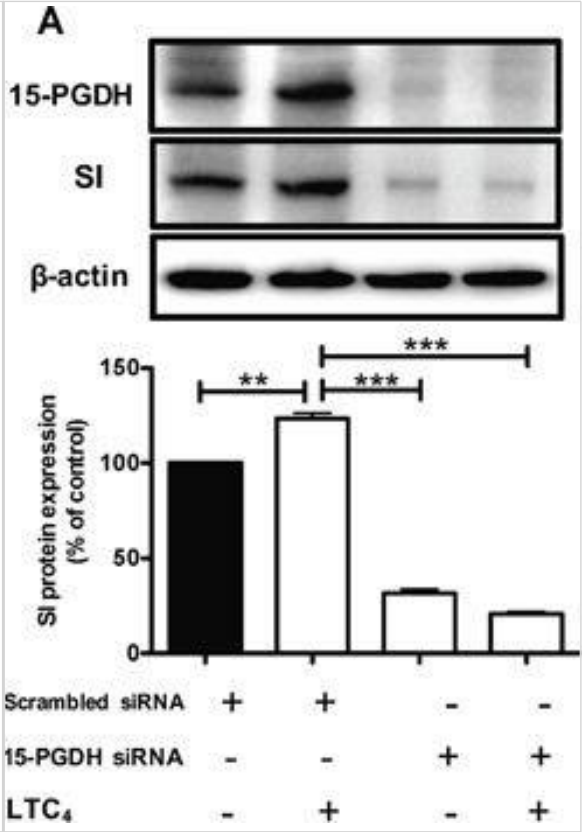
Simple Western: 15-PGDH/HPGD Antibody [NB200-179] - Simple Western lane view shows a specific band for 15-PGDH/HPGD in 0.5 mg/ml of Lovo (left) and HeLa (right) lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



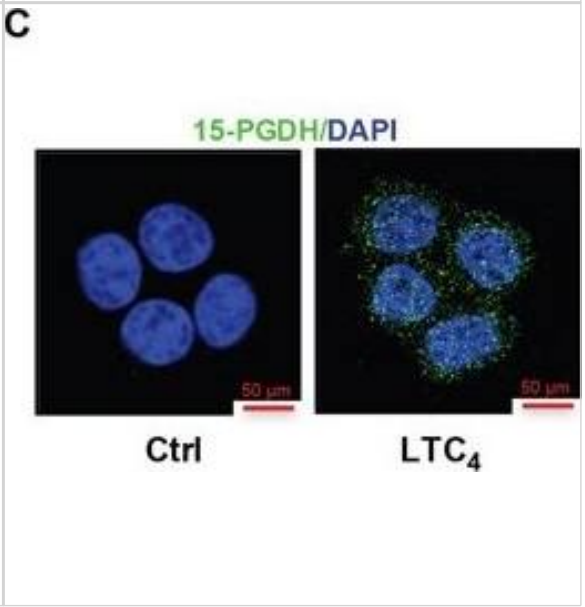
Immunohistochemistry: 15-PGDH/HPGD Antibody [NB200-179] - Expression of 15-prostaglandin dehydrogenase (15-PGDH) in healthy and inflamed synovial tissue. Immunohistochemical staining reveals positive (brown) staining for 15-PGDH (hematoxilin counterstained) in synovial tissue from healthy individuals (a) and patients with rheumatoid arthritis (b), osteoarthritis (c), or psoriatic arthritis (d). Original magnifications: x250 (a, b), x100 (c, d). Image collected and cropped by CiteAb from the following publication (https://arthritis-research.biomedcentral.com/articles/10.1186/ar3851), licensed under a CC-BY license.	
Immunohistochemistry: 15-PGDH/HPGD Antibody [NB200-179] - Cellular localization of 15-prostaglandin dehydrogenase (15-PGDH/HPGD) in rheumatoid arthritis synovial tissue and the effects of anti-rheumatic treatment on its expression. Double-immunofluorescence images show staining for 15-PGDH/HPGD-positive (red) cells and cell marker staining (green) for macrophage CD163, fibroblast prolyl-4-hydroxylase, and endothelial cell CD31. Merged images display double-stained cells in yellow. Original magnification: x400. Image collected and cropped by CiteAb from the following publication (https://arthritis-research.biomedcentral.com/articles/10.1186/ar3851), licensed under a CC-BY license	
Western Blot: 15-PGDH/HPGD Antibody [NB200-179] - Western blot analysis of 15-PGDH in Lovo cell lysate.	
Immunocytochemistry/Immunofluorescence: 15-PGDH/HPGD Antibody [NB200-179] - Confocal microscopy immunofluorescence images showing the expression of 15-PGDH (in green; antibody dilution, 1:200; DAPI in blue) after 24 h of stimulation with LTC4 in Caco-2 cells. The objective used was 63x. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/28402256/) licensed under a CC-BY license.	

Immunocytochemistry/Immunofluorescence: 15-PGDH/HPGD Antibody [NB200-179] - 15-PGDH antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).	
Immunohistochemistry-Paraffin: 15-PGDH/HPGD Antibody [NB200-179] - IHC analysis of 15-PGDH in human synovial tissue from patient with rheumatoid arthritis . Image courtesy of an anonymous customer review.	
Immunohistochemistry: 15-PGDH/HPGD Antibody [NB200-179] - Staining in human fetal membrane tissue. Image from verified customer review.	
Knockdown Validated: 15-PGDH/HPGD Antibody [NB200-179] - A representative western blot and densitometric analysis of LTC4-induced SI protein expression after transfection with a scrambled control siRNA or 15-PGDH siRNA in HT-29 cells are shown. The cells were treated with or without 40 nM LTC4 for 48 h, and the change in the SI protein level was detected using an SI-specific antibody (1:1000 dilution) and 15-PGDH was detected using a 15-PGDH antibody (1:5000 dilution). The membrane was re-probed with an antibody against beta-actin to ensure equal loading. Statistical analysis was conducted using an unpaired t-test; *P=0.05, **P<0.01. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/28402256/) licensed under a CC-BY license.	

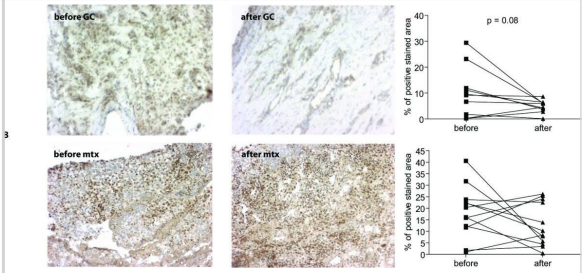
G-TPP leads to recruitment of autophagy adapters and degradation of mitochondria(A) HeLa cells stably expressing untagged Parkin were treated with 10 μM G-TPP for 8 h. Western blots were prepared from cell lysates and probed with antibodies against LC3, phospho-TBK1 (Ser172) and TBK1. GAPDH was used as a loading control. Upon 8 h the levels of LC3-I and LC3-II were both increased. At 8 h after treatment with G-TPP but not at 4 or 24 h, TBK1 was phosphorylated. (B) HeLa cells stably expressing EGFP-Parkin were treated with 10 μM G-TPP and fixed 8 h after treatment. Cells were stained with antibodies against the autophagy adapter proteins NBR1, NDP52, OPTN, p62, and TAX1BP1 (red). Mitochondria were counterstained with TOM20 antibodies (cyan), nuclei with Hoechst (blue). EGFP-Parkin epifluorescence is shown in green. Scale bar corresponds to 10 μM. (C) HeLa cells stably expressing EGFP-Parkin and the reporter protein mitoKeima were treated with 10 μM CCCP or G-TPP and imaged over time. The ratio of 'neutral' mitoKeima to 'acidic' mitoKeima was calculated as readout for mitophagy. Parkin translocation was monitored at the same time. Values for Parkin translocation and mitophagy were normalized to 12 h treatment with 10 μM CCCP as positive control and DMSO as negative control (two-way ANOVA with Tukey's post-hoc test, **p < 0.005, ***p < 0.0005). Image collected and cropped by CiteAb from the following open publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.22287), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



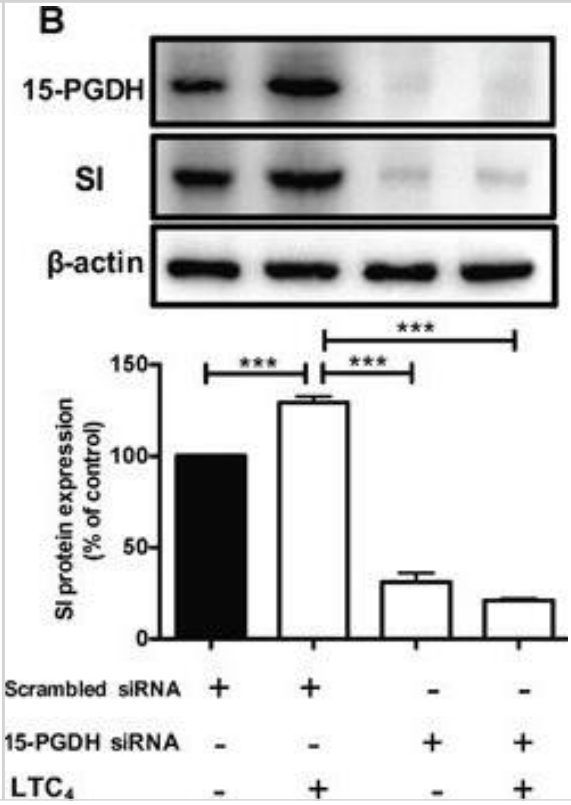
Effects of oltipraz on the expression of CD11b/c, phosphoinositide 3-kinase (PI3K), phosphorylated protein kinase B (p-Akt), and phosphorylated inhibitor of κBα (p-IkBα) in the spinal cord of the CCI-injured mice. The relative protein levels of (A) CD11b/c, (B) PI3K, (D) p-Akt, and (E) p-IKBα on the ipsilateral side of the spinal cord in the CCI-injured mice treated with oltipraz (OLT) or vehicle are represented. The sham-operated mice (SHAM) treated with vehicle were used as controls. (C) Representative examples of blots for CD11b/c (160 kDa), PI3K (130 kDa), and GAPDH (37 kDa), and (F) for p-Akt (60 kDa), Akt (60 kDa), p-IKBα (40 kDa) and IKBα (40 kDa). CD11b/c and PI3K are expressed relative to GAPDH levels whereas phosphorylated proteins are expressed relative to their corresponding total proteins. In all panels, * denotes significant differences vs. sham-operated mice treated with vehicle (p < 0.05; one-way ANOVA followed by the SNK test). Results are presented as the mean ± SEM; n = 5 samples per experimental group. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/31234342), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



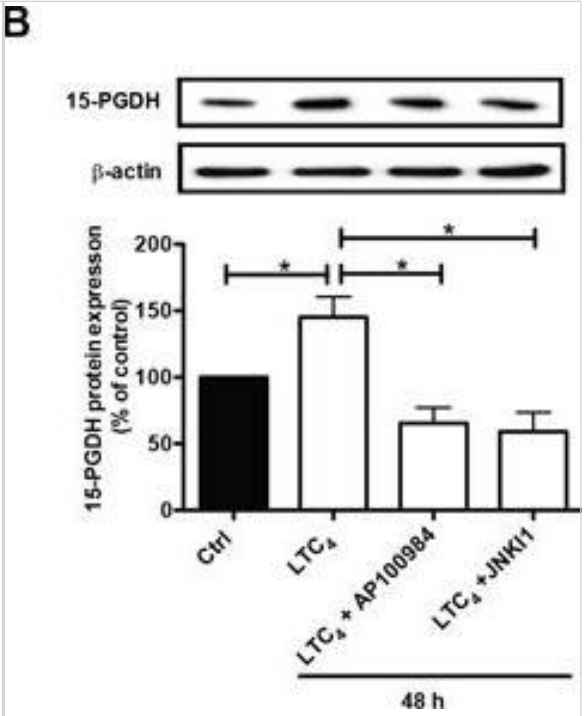
Normoxic pretreatment sensitizes glioma cells to radiation after rapid acute hypoxic (RAH) exposure.(A) The rapid acute hypoxia (RAH) protocol is shown depicting the timing and severity of hypoxic exposure. Cells either remain in a continuous hypoxic environment (–) or are transiently (25 min) exposed to normoxia 25 min prior to radiation (+). Continuously normoxic cells (NOx) were irradiated as a positive control. (B) The results of anchorage-independent colony forming assays are shown for U87, U87-luc, GL261 glioma cells and 0308 GSCs after 5 Gy radiation exposure under varying oxygen conditions. To allow for ease of comparisons among cell types, raw values are expressed as a percentage of the corresponding cell type's negative (non-irradiated) control and the means and SEMs are plotted. Each result represents at least three independent samples, plated in triplicate. Holm-Sidak comparisons for multiple groups were used for statistical comparisons of raw values (**p<0.01). Also shown are Western blots of nuclear HIF-1α at the time of irradiation for each cell type. Corresponding Western blots of lamin A/C are shown as a loading control. All lanes shown that are non-adjacent to the negative control (NOx) are denoted with a separating black line. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/25350400), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



α5β1 regulates ED-A FN secretion and polymerization.(a) Western blot analysis of lysates of ECs control (siCTL) and α5 integrin subunit (siITGA5) silenced ECs. Cells were lysed 24 hours after the second siRNA oligofection and proteins were separated by SDS–PAGE and probed for α5 integrin subunit or actin (for control purposes). (b) Confocal microscopy analysis of IST9 mAb-labelled endogenous ED-A FN (green) in confluent ECs. ED-A FN polymerizes into a fibrillar network in siCTL, but not in siITGA5 ECs in which it accumulates in the TGN46+ (red) TGN cisternae. The relative amount of fibrillar ED-A FN area was calculated in siCTL and siITGA5 ECs. Data are mean±s.e.m., n=20 cells per condition pooled from two independent experiments. ***P<0.001; Student's t-test. (c) Western blot analysis of soluble ED-A FN released by confluent ECs seeded on Transwell inserts. An equal percentage of apical and basolateral volumes of medium were collected after 72 h of culture, from different wells of siCTL or siITGA5 ECs. Equal amounts of exogenous rabbit IgG were added to samples (spike normalization) for loading control purposes. Quantification of the ratio between apical or basolateral amount of ED-A FN released by siCTL over siITGA5 ECs. α5 integrin subunit silencing impairs basolateral, but not apical ED-A FN secretion. Data are mean±s.e.m., n=8 wells per condition pooled from four independent experiments. **P<0.01; Student's t-test. Scale bar, 50 μm (b). Image collected and cropped by CiteAb from the following open publication (https://www.nature.com/articles/ncomms13546), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Phase contrast microscopy images of HFA and brain tumor cells and immunofluorescence images of cultured primary brain tumor cells, AA and HFA.HFA were derived from three different foetal brains. (A) 18-week foetus (10× magnification); (B) 19-week foetus, first week in culture (10× magnification); (C) 19-week foetus, day 5 in culture (20× magnification); (D) Primary GBM after 2 trypsinisations (20x); (E) Recurrent GBM (20x); (F) Secondary GBM after 3 trypsinisations (10x); (G) OII 14 days in culture (10x); (H) AII 13 days in culture (10x); (I) Negative control for NGS merged with DAPI; (J) Negative control for secondary antibodies merged with DAPI; (K) GBM cells derived from one patient – GFAP (DAKO) merged with DAPI and CD68 (zoomed: 2.4× magnification); (L) primary brain tumor cells from one patient (male, 40 years old) (passaged three times) - GFAP (Novocastra) merged with DAPI; (K and L) GFAP (green) was used as a GBM marker and CD68 (red) was used as a marker for microglia. (M) AA from one Female, 61 years old; (passaged twice) - GFAP (Novocastra) merged with DAPI; (N) AA from one male 40 years old; (passaged once) - GFAP (Sigma) merged with DAPI and CD68 (Abcam); (M and N) GFAP (green) was used as an astrocyte marker and CD68 (red) and CD11b (green) were used as markers for microglia; (O) HFA from one 17-week- old foetus (passaged once) - GFAP (Novocastra) merged with DAPI and CD11b (Novus); (P) HFA from an 18-week- old foetus (passaged once) - GFAP (DAKO) merged with DAPI and CD68 (zoomed: 2.1× magnification); (O and P) GFAP (green and red) was used as an astrocyte marker and CD68 (red) and CD11b (green) were used as markers for microglia. Nuclei indicated by DAPI (blue) in all images. Image collected and cropped by CiteAb from the following open publication (https://dx.plos.org/10.1371/journal.pone.0112945), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Ghatak S, Mehrabi SF, Mehdawi LM et al. Identification of a Novel Five-Gene Signature as a Prognostic and Diagnostic Biomarker in Colorectal Cancers International journal of molecular sciences 2022-01-12 [PMID: 35054980] (IHC-P, Human)

Loo T M, Kamachi F et al. Gut Microbiota Promotes Obesity-Associated Liver Cancer through PGE2-Mediated Suppression of Antitumor Immunity. Cancer Discov 2017-01-05 [PMID: 28202625] (WB, Mouse)

Volpato M, Cummings M, Shaaban A et al. Downregulation of 15-hydroxyprostaglandin dehydrogenase during acquired tamoxifen resistance and association with poor prognosis in ER alpha-positive breast cancer Exploration of Targeted Anti-tumor Therapy 2020-10-19 [PMID: 33210098] (Human)

Osman J CysLT1 receptor signaling and the tumor microenvironment in colon cancer models Thesis 2020-01-01

Corwin C, Nikolopoulou A, Pan AL et al. Prostaglandin D2/J2 signaling pathway in a rat model of neuroinflammation displaying progressive parkinsonian-like pathology: potential novel therapeutic targets. J Neuroinflammation 2018-09-20 [PMID: 30236122] (ICC/IF, Rat)

Mehdawi LM, Satapathy SR, Gustafsson A et al. A potential anti-tumor effect of leukotriene C4 through the induction of 15-hydroxyprostaglandin dehydrogenase expression in colon cancer cells. Oncotarget. 2017-05-23 [PMID: 28402256] (IF/IHC, ICC/IF, Human)

Yao L, Chen W, Song K et al. 15-hydroxyprostaglandin dehydrogenase (15-PGDH) prevents lipopolysaccharide (LPS)-induced acute liver injury. PLoS ONE. 2017-04-19 [PMID: 28423012] (IHC-P, Mouse)

Prima V, Kaliberova LN, Kaliberov S et al. COX2/mPGES1/PGE2 pathway regulates PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells Proc. Natl. Acad. Sci. U.S.A 2017-01-17 [PMID: 28096371] (WB, Mouse)

Mehdawi LM, Prasad CP, Ehrnstrom R et al. Non-canonical WNT5A signaling up-regulates the expression of the tumor suppressor 15-PGDH and induces differentiation of colon cancer cells. Mol Oncol. 2016-08-01 [PMID: 27522468] (WB, IF/IHC)

Miyagishi H, Kosuge Y, Takano A et al. Increased Expression of 15-Hydroxyprostaglandin Dehydrogenase in Spinal Astrocytes During Disease Progression in a Model of Amyotrophic Lateral Sclerosis. Cell. Mol. Neurobiol. 2016-05-02 [PMID: 27140190] (IHC-Fr, WB, Mouse)

Hsiao HM, Thatcher TH, Colas RA et al. Resolvin D1 Reduces Emphysema and Chronic Inflammation. Am J Pathol 2015-12-01 [PMID: 26468975]

de Hair MJ, Leclerc P, Newsum EC et al. Expression of Prostaglandin E2 Enzymes in the Synovium of Arthralgia Patients at Risk of Developing Rheumatoid Arthritis and in Early Arthritis Patients. PLoS ONE. 2015-08-03 [PMID: 26225917] (IF/IHC, Human)

More publications at http://www.novusbio.com/NB200-179

Procedures

Western Blot Protocols specific for 15-PGDH Antibody (NB200-179)

Western Blot I (LoVo lysates)

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 20ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transferapparatus.
3. 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% non-fat dry milk in TBS for 1 hour.
6. Dilute the rabbit anti-15-PGDH primary antibody (NB 200-179) in blocking buffer and incubate overnight at 4C.
7. Wash the membrane in water for 5 minutes and apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
8. Wash the blot in TBS containing 0.05-0.1% Tween-20 for 10-20 minutes.
9. Wash the blot in type I water for an additional 10-20 minutes (this step can be repeated as required to reduce background).
10. Apply the detection reagent of choice in accordance with the manufacturers instructions (Amersham's ECL is the standard reagent used at Novus Biologicals).

**Note: Tween-20 can be added to the blocking buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Western Blot II (A549 lysates)

1. Cell lysates are prepared in RIPA buffer (50 mM 50 mM Tris-HCL/1% Nonidet P-40/0.25% Na-deoxycholate/150 mM NaCl/1 mM EDTA/1 mM PMSF) supplemented with a protease inhibitor mixture (Roche Applied Sciences).
2. They are then separated on 10% or 12% SDS/PAGE (30-150 ug per lane) and transferred to a Immobilon PVDF membrane (Millipore).
3. The membrane is blocked with 5% NFDM in TBS-T (TBS + 0.1% Tween-20).
4. The membrane is then probed with the diluted anti-PGDH antibody (NB 200-179), diluted in blocking buffer at RT for 1 hour.
5. The membrane is washed 3 times with TBS-T.
6. The membrane is then incubated with a biotinylated goat anti-rabbit IgG (diluted as per manufacturer's guidelines in blocking buffer) at RT for 1 hour.
7. The membrane was washed extensively.
8. The membrane is then incubated with an HRP-conjugated streptavidin (1:2,000) complex at RT for 1 hour.
9. The membrane is washed extensively.
10. The membrane is developed using and ECL detection system.

Immunocytochemistry/Immunofluorescence protocol for 15-PGDH/HPGD Antibody (NB200-179)

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Immunohistochemistry paraffin-embedded protocol (NB200-179)

Immunohistochemistry

1. 5 uM-thick formalin-fixed paraffin-embedded tissue sections were baked at 60C for 75 minutes, deparaffinized, and rehydrated.
2. Antigen retrieval was performed by steaming the sections at 96C for 5 minutes in 10 mM citrate buffer (pH 6.0), plus a cool-down period of 20 minutes.
3. Reduction of peroxidases was accomplished by incubating in 3% H2O2 in water for 30 minutes at room temperature.
4. Avidin ??biotin blocking was performed for 15 minutes each, followed by nonspecific protein blocking (Serum-Free Protein Block, Dako, Carpenteria, CA) performed for 60 minutes.
5. Primary antibody was diluted in 1% BSA and incubated overnight at 4C in humidified chambers.
6. The slides were washed thoroughly, and Protein Block was added again for 30 minutes.
7. LSAB+ anti-rabbit kit (Dako) was used for development, applying the secondary antibody and HRP-conjugated streptavidin per the manufacturer's instructions.
8. Diaminobenzidine (Dako) was added to the slides for 10 minutes.
9. The sections were then counterstained by using Harris modified hematoxylin stain (Fisher Scientific) for 1 minute.

**Note: All washes were done with TBS (50 mM Tris-HCl/150 mM NaCl, pH 7.6) diluted in deionized water.

Novus Biologicals USA
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

Products Related to NB200-179

NBL1-11693	15-PGDH/HPGD Overexpression Lysate
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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NB200-179

Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications