

# Product Datasheet

## 15-PGDH/HPGD Antibody - BSA Free 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](http://www.novusbio.com)



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**NB200-179**

15-PGDH/HPGD Antibody - BSA Free

**Product Information**

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

**Product Description**

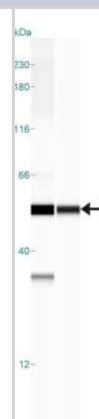
<b>Host</b>	Rabbit
<b>Gene ID</b>	3248
<b>Gene Symbol</b>	HPGD
<b>Species</b>	Human, Mouse, Rat
<b>Reactivity Notes</b>	Mouse reactivity reported in scientific literature (PMID: 27140190) Human reactivity reported in scientific literature (PMID:33210098).
<b>Immunogen</b>	Purified type I human placental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

**Product Application Details**

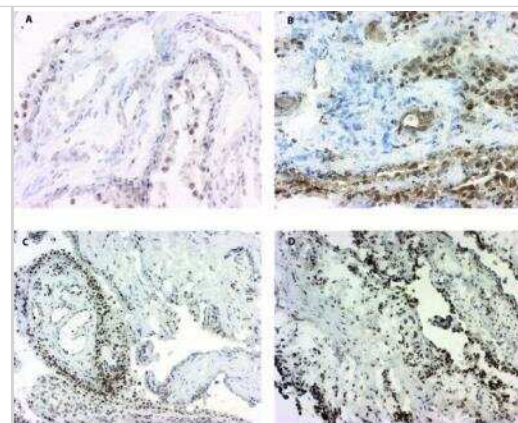
<b>Applications</b>	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Knockdown Validated
<b>Recommended Dilutions</b>	Western Blot 1:5000-1:6000, Simple Western 1:500, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen, Knockdown Validated

**Images**

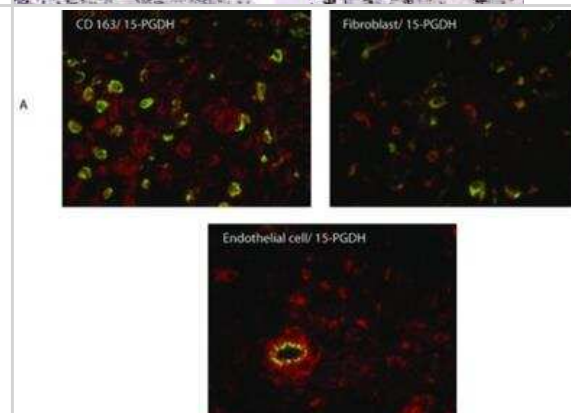
Simple Western: 15-PGDH/HPGD Antibody [NB200-179] - Simple Western lane view shows a specific band for 15-PGDH/HPGD in 0.5 mg/ml of Lovo (left) and HeLa (right) lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



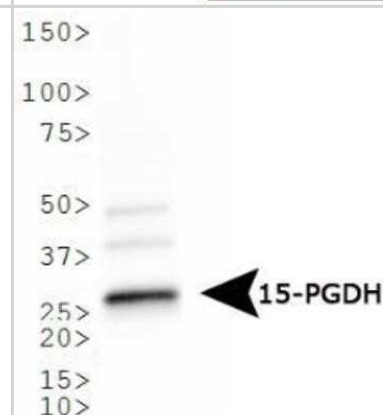
**Immunohistochemistry: 15-PGDH/HPGD Antibody [NB200-179]** - Expression of 15-prostaglandin dehydrogenase (15-PGDH) in healthy and inflamed synovial tissue. Immunohistochemical staining reveals positive (brown) staining for 15-PGDH (hematoxylin counterstained) in synovial tissue from healthy individuals (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.



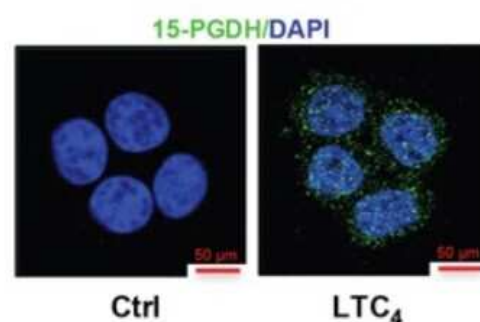
**Immunohistochemistry: 15-PGDH/HPGD Antibody [NB200-179]** - Cellular localization of 15-prostaglandin dehydrogenase (15-PGDH/HPGD) in rheumatoid arthritis synovial tissue and the effects of anti-rheumatic treatment on its expression. Double-immunofluorescence images show staining for 15-PGDH/HPGD-positive (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



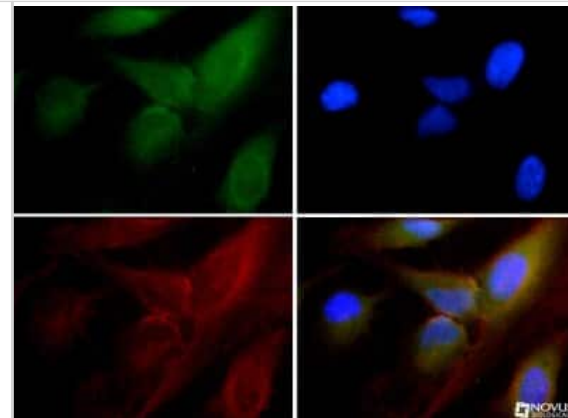
**Western Blot: 15-PGDH/HPGD Antibody [NB200-179]** - Western blot analysis of 15-PGDH in Lovo cell lysate.



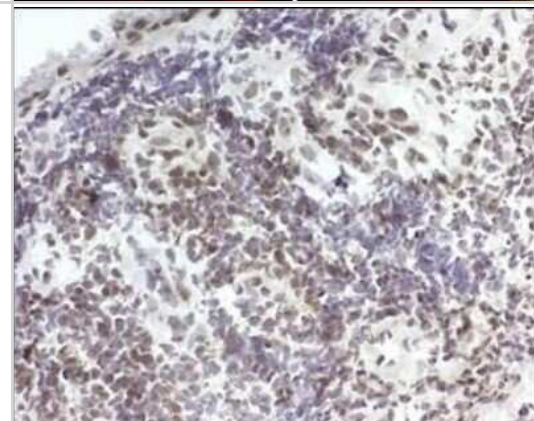
**Immunocytochemistry/Immunofluorescence: 15-PGDH/HPGD Antibody [NB200-179]** - Confocal microscopy immunofluorescence images showing the expression of 15-PGDH (in green; antibody dilution, 1:200; DAPI in blue) after 24 h of stimulation with LTC<sub>4</sub> in Caco-2 cells. The objective used was 63x. Image collected and cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28402256/>) licensed under a CC-BY license.



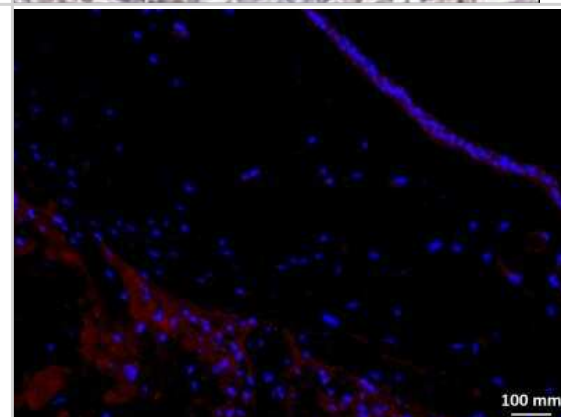
**Immunocytochemistry/Immunofluorescence: 15-PGDH/HPGD Antibody [NB200-179]** - 15-PGDH antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



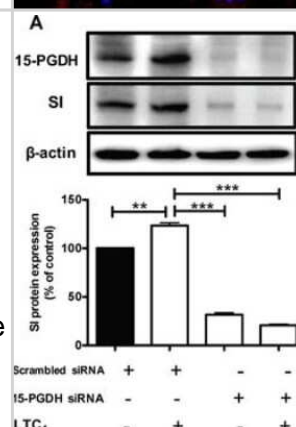
**Immunohistochemistry-Paraffin: 15-PGDH/HPGD Antibody [NB200-179]** - IHC analysis of 15-PGDH in human synovial tissue from patient with rheumatoid arthritis. Image courtesy of an anonymous customer review.



**Immunohistochemistry: 15-PGDH/HPGD Antibody [NB200-179]** - Staining in human fetal membrane tissue. Image from verified customer review.

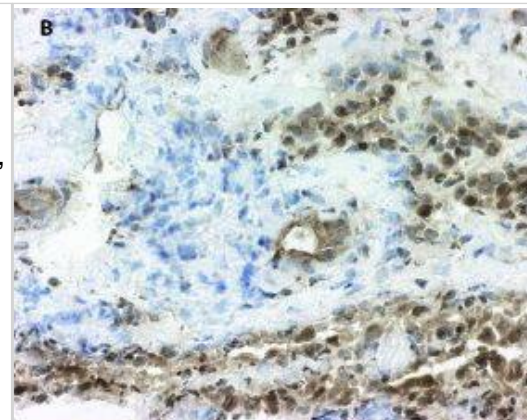


**Western Blot: 15-PGDH/HPGD Antibody [NB200-179]** - A representative western blot and densitometric analysis of LTC<sub>4</sub>-induced SI protein expression after transfection with a scrambled control siRNA or 15-PGDH siRNA in HT-29 cells are shown. The cells were treated with or without 40 nM LTC<sub>4</sub> 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 (<https://pubmed.ncbi.nlm.nih.gov/28402256/>) licensed under a CC-BY license.

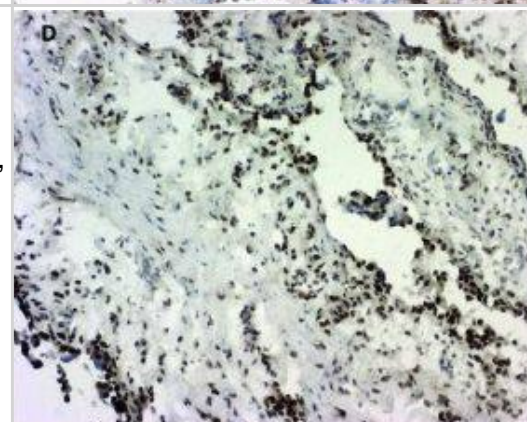




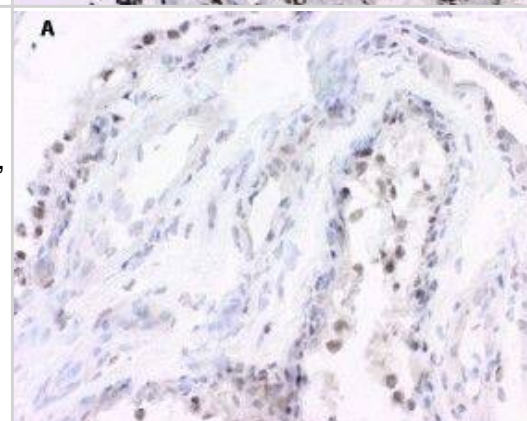
Immunohistochemistry: 15-PGDH/HPGD Antibody [NB200-179] - Expression of 15-prostaglandin dehydrogenase (15-PGDH) in healthy & inflamed synovial tissue. Immunohistochemical staining reveals positive (brown) staining for 15-PGDH (hematoxylin counterstained) in synovial tissue from healthy individuals (a) & patients with rheumatoid arthritis (b), osteoarthritis (c), or psoriatic arthritis (d). Original magnifications:  $\times 250$  (a, b),  $\times 100$  (c, d). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/22616846>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



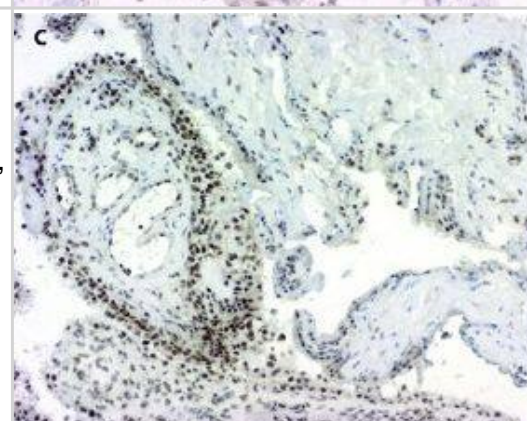
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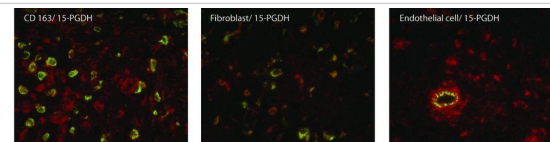
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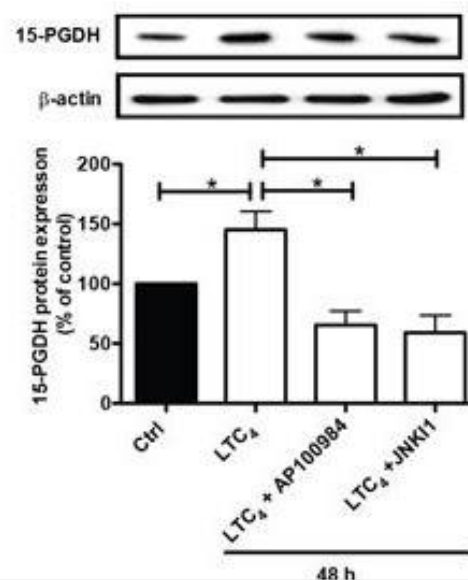


**Immunocytochemistry/ Immunofluorescence: 15-PGDH/HPGD Antibody [NB200-179]** - Cellular localization of 15-prostaglandin dehydrogenase (15-PGDH) in rheumatoid arthritis synovial tissue & the effects of anti-rheumatic treatment on its expression. (a) Double-immunofluorescence images show staining for 15-PGDH-positive (red) cells & cell marker staining (green) for macrophage CD163, fibroblast prolyl-4-hydroxylase, & endothelial cell CD31. Merged images display double-stained cells in yellow. Original magnification:  $\times 400$ . Light microscopy pictures of representative synovial biopsy sections show immunohistochemical positive (brown) staining for 15-PGDH before & after intra-articular treatment with glucocorticoids (b) & before & 8 weeks after initiation of methotrexate therapy (c) (hematoxylin counterstained). Graphs display the comparative 15-PGDH expression in rheumatoid arthritis synovial tissue before & after treatment with glucocorticoids or methotrexate as a percentage of the positive stained area versus the total tissue area. GC, glucocorticoids; Mtx, methotrexate. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/22616846>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



**Western Blot: 15-PGDH/HPGD Antibody [NB200-179]** - Effect of LTC<sub>4</sub> on 15-PGDH expression in the colon cancer cell line Caco-2(A) Quantification by qPCR of the mRNA expression of 15-PGDH following treatment with 40 nM LTC<sub>4</sub> for 48 h in the presence or absence of AP100984 (a CysLT<sub>2</sub> receptor antagonist, 1  $\mu$ M) & JNK inhibitor I (10  $\mu$ M). (B) Western blot showing 15-PGDH expression (antibody dilution, 1:5000) & densitometric analysis of LTC<sub>4</sub>-induced 15-PGDH up-regulation before or after the cells were stimulated with 40 nM LTC<sub>4</sub> in the presence or absence of AP100984 & JNK inhibitor I for 48 h. (C) 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 LTC<sub>4</sub> in Caco-2 cells. The objective used was 63x. The data are presented as the percent of untreated control cells & represent the mean  $\pm$  SEM of at least three separate experiments. Statistical analysis was conducted using an unpaired t-test; \* $P \leq 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28402256>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

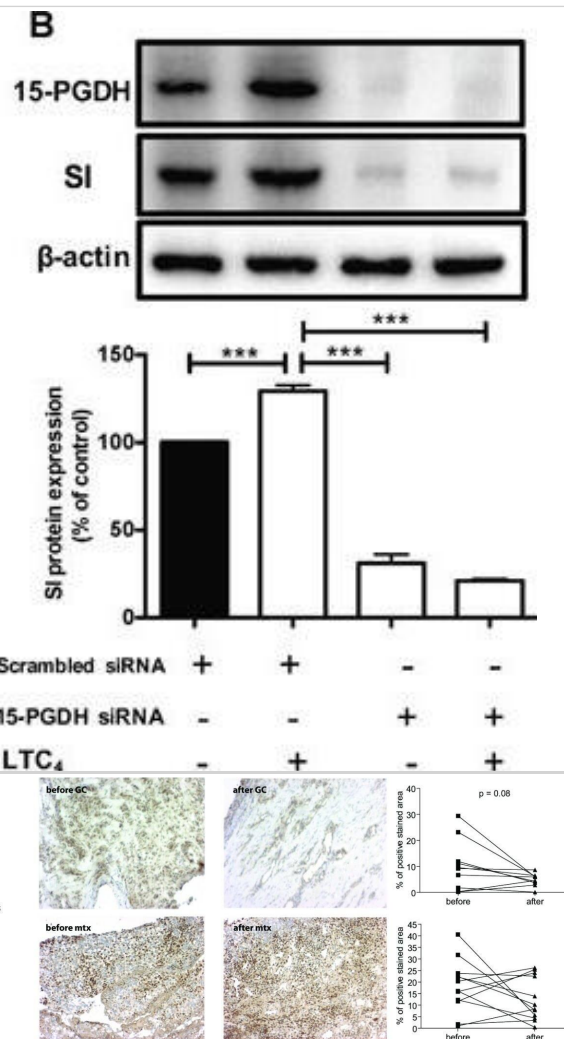
B





**Western Blot: 15-PGDH/HPGD Antibody [NB200-179] - Effect of 15-PGDH down-regulation on the cell differentiation markers SI & Mucin-2**

(A) A representative western blot & densitometric analysis of LTC<sub>4</sub>-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 LTC<sub>4</sub> for 48 h, & the change in the SI protein level was detected using an SI-specific antibody (1:1000 dilution) & 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. (B) A representative western blot & densitometric analysis performed as in (A) shown here for Caco-2 cells. (C, D) Representative confocal microscopy immunofluorescence images from cells transfected with a scrambled control siRNA or 15-PGDH siRNA with or without stimulation with 40 nM LTC<sub>4</sub> for 48 h. The expression of Mucin-2 (in red; antibody dilution, 1:500; DAPI in blue, dilution 1:1000) (C) in HT-29 cells & (D) Caco-2 cells is shown. The objective used was 63x, & the scale bar is 50  $\mu$ m. The data are presented as the percent of control cells & represent the mean  $\pm$  SEM of at least three separate experiments. Statistical analysis was conducted using an unpaired t-test; \*P $\leq$ 0.05, \*\*P $\leq$ 0.01. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28402256>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



**Immunohistochemistry: 15-PGDH/HPGD Antibody [NB200-179] - Cellular localization of 15-prostaglandin dehydrogenase (15-PGDH) in rheumatoid arthritis synovial tissue & the effects of anti-rheumatic treatment on its expression.**

(a) Double-immunofluorescence images show staining for 15-PGDH-positive (red) cells & cell marker staining (green) for macrophage CD163, fibroblast prolyl-4-hydroxylase, & endothelial cell CD31. Merged images display double-stained cells in yellow. Original magnification:  $\times$ 400. Light microscopy pictures of representative synovial biopsy sections show immunohistochemical positive (brown) staining for 15-PGDH before & after intra-articular treatment with glucocorticoids (b) & before & 8 weeks after initiation of methotrexate therapy (c) (hematoxylin counterstained). Graphs display the comparative 15-PGDH expression in rheumatoid arthritis synovial tissue before & after treatment with glucocorticoids or methotrexate as a percentage of the positive stained area versus the total tissue area. GC, glucocorticoids; Mtx, methotrexate. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/22616846>), 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 transfer apparatus.
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 1:25,000 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  $\mu$ m-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% H<sub>2</sub>O<sub>2</sub> 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, Carpinteria, 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.





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General: novus@novusbio.com

### **Products Related to NB200-179**

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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

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### **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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