

Product Datasheet

Pyruvate Carboxylase Antibody - BSA Free NBP1-49536

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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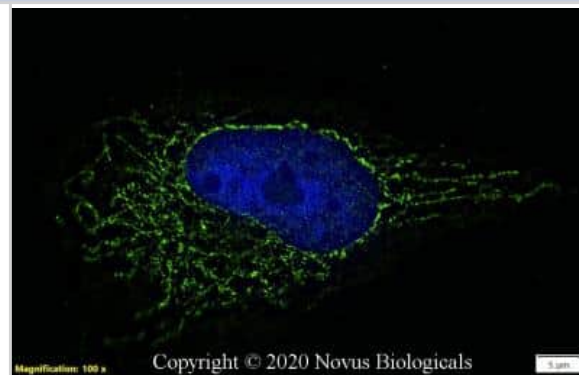


NBP1-49536**Pyruvate Carboxylase Antibody - BSA Free**

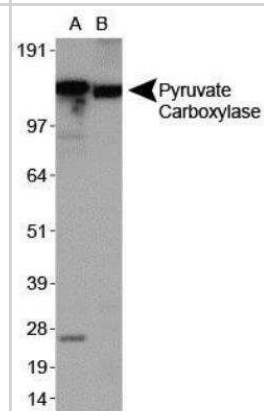
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	126 kDa
Product Description	
Description	Novus Biologicals Rabbit Pyruvate Carboxylase Antibody - BSA Free (NBP1-49536) is a polyclonal antibody validated for use in IHC, WB and ICC/IF. Anti-Pyruvate Carboxylase Antibody: Cited in 17 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	5091
Gene Symbol	PC
Species	Human, Mouse, Rat
Reactivity Notes	Immunogen has 96% identity to bovine and porcine. Rat reactivity reported in scientific literature (PMID: 24333689)
Immunogen	Partial recombinant protein made to an internal region of the human Pyruvate Carboxylase protein (within residues 930-1050). [Swiss-Prot P11498]
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Knockdown Validated
Recommended Dilutions	Western Blot 1:1000. Use reported in scientific literature (PMID 29937374), Immunohistochemistry 1:200, Immunocytochemistry/Immunofluorescence 1:50-1:100, Immunohistochemistry-Paraffin 1:200, Knockdown Validated
Application Notes	In Western blot, a band is seen at ~129 kDa. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Images

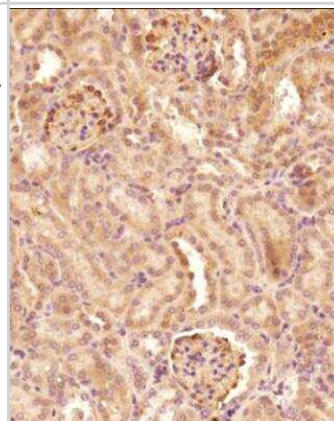
Immunocytochemistry/Immunofluorescence: Pyruvate Carboxylase Antibody [NBP1-49536] - HeLa cells were fixed in 4% paraformaldehyde for 10 min and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti- (NBP1-49536) at 10 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



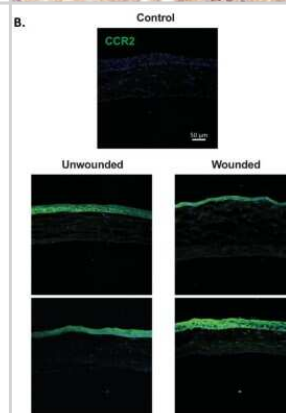
Western Blot: Pyruvate Carboxylase Antibody [NBP1-49536] - Analysis of Pyruvate Carboxylase in A. Human liver extracts and B. Mouse liver extracts



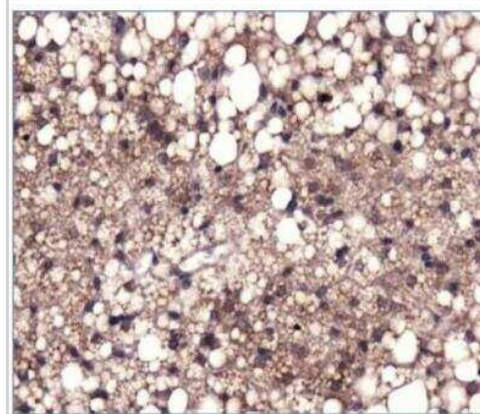
Immunohistochemistry-Paraffin: Pyruvate Carboxylase Antibody [NBP1-49536] - IHC analysis of a formalin fixed and paraffin embedded tissue section of mouse kidney using rabbit anti-Pyruvate Carboxylase antibody at 1:200 dilution. The signal was developed using HRP conjugated anti-rabbit secondary antibody and DAB reagent. The nuclei were counterstained using hematoxylin. This Pyruvate Carboxylase antibody generated a diffused cytoplasmic staining in all the tubules and a subset of cells in glomerular cells.



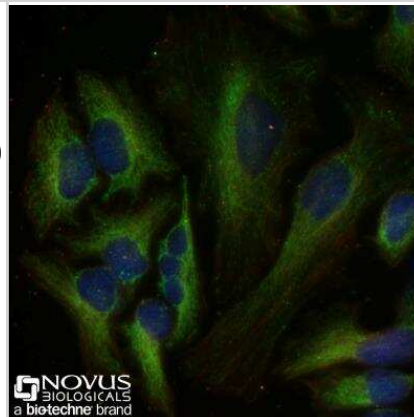
Western Blot: Pyruvate Carboxylase Antibody [NBP1-49536] - Characterization of MPC1^{-/-} in the RM-1 murine prostate cancer cells. Western blotting results of subcellular proteins from WT and MPC1^{-/-} cells and corresponding densitometry histograms of ALT1 and Pyruvate Carboxylase. Cyto means cytoplasmic protein. Mito stands for mitochondrial protein. Protein alpha-Tubulin was used as a loading control. ***P<0.001. All experiments were performed at least three times with consistent and repeatable results. Image collected and cropped by CiteAb from the following publication (<https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.18199>), licensed under a CC-BY license.



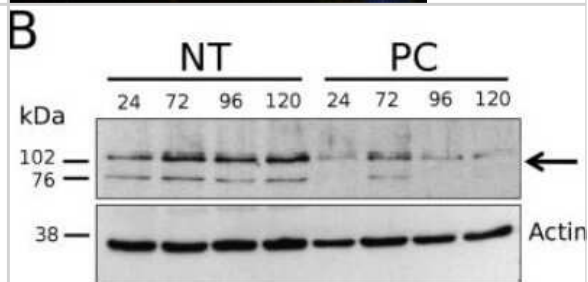
Immunohistochemistry-Paraffin: Pyruvate Carboxylase Antibody [NBP1-49536] - IHC analysis of a formalin fixed and paraffin embedded tissue section of mouse adipose tissue using rabbit anti-Pyruvate Carboxylase antibody at 1:200 dilution. The signal was developed using HRP conjugated anti-rabbit secondary antibody and DAB reagent. The nuclei were counterstained using hematoxylin. This Pyruvate Carboxylase antibody generated a specific staining of the fat cells in the tested section.



Immunocytochemistry/Immunofluorescence: Pyruvate Carboxylase Antibody [NBP1-49536] - HeLa cells were fixed and permeabilized for 10 minutes using -20C MeOH. The cells were incubated with anti-Pyruvate Carboxylase 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.



Western Blot: Pyruvate Carboxylase Antibody [NBP1-49536] - HSV-1 replication is inhibited by reducing flux from glucose toward pyrimidine nucleotide synthesis. RNA interference knockdown of pyruvate carboxylase (marked by arrow) in MRC5 cells. Cells were transfected with non-targeting siRNAs (NT) or siRNAs targeting pyruvate carboxylase (PC) and harvested at indicated time points after transfection. Pyruvate carboxylase levels in the cells were detected by western blot using specific antibodies. Beta-actin was employed as a loading control. Divergent effects of human cytomegalovirus and herpes simplex virus-1 on cellular metabolism. PLoS Pathog (2011)



Publications

Shankar TS, Ramadurai DKA, Steinhorst K et al. Cardiac-specific deletion of voltage dependent anion channel 2 leads to dilated cardiomyopathy by altering calcium homeostasis Nature Communications 2021-07-28 [PMID: 34321484] (Western Blot, Human)

Igelmann S, Lessard F, Uchenunu O et al. A hydride transfer complex reprograms NAD metabolism and bypasses senescence Molecular cell 2021-09-16 [PMID: 34547241]

Kershberg L, Banerjee A, Kaeser PS. Protein composition of axonal dopamine release sites in the striatum eLife 2022-12-29 [PMID: 36579890] (Western Blot, Immunofluorescence, Immunocytochemistry)

Xin Cai, Charles P Ng, Olivia Jones, Tak Shun Fung, Keun Woo Ryu, Dayi Li, Craig B Thompson Lactate activates the mitochondrial electron transport chain independently of its metabolism. Molecular cell 2023-11-06 [PMID: 37879334]

Guo X, Jiang X, Chen K et al. The Role of Palmitoleic Acid in Regulating Hepatic Gluconeogenesis through SIRT3 in Obese Mice Nutrients 2022-04-01 [PMID: 35406095] (WB, Mouse)

Chung CY, Singh K, Kotiadis VN Et al. Constitutive activation of the PI3K-Akt-mTORC1 pathway sustains the G mtDNA mutation Nature communications 2021-11-04 [PMID: 34737295] (WB, Human)

Schworer S, Pavlova NN, Cimino FV Et al. Fibroblast pyruvate carboxylase is required for collagen production in the tumour microenvironment Nature metabolism 2021-11-01 [PMID: 34764457] (WB, Human)

Calvete O, Reyes J, Benitez J Case Report: CMV Infection and Same Mechanism-Originated Intestinal Inflammation Compatible With Bowel/Crohn's Disease Is Suggested in ATP4A Mutated-Driven Gastric Neuroendocrine Tumors Frontiers in medicine 2021-04-06 [PMID: 33889580] (IF/IHC, Human)

Song X, Liu J, Kuang F et al. PDK4 dictates metabolic resistance to ferroptosis by suppressing pyruvate oxidation and fatty acid synthesis Cell reports 2021-02-23 [PMID: 33626342]

Chung C, Singh K, Kotiadis V et al. Maladaptive nutrient signalling sustains the m.3243A>G mtDNA mutation bioRxiv 2020-06-19 (WB, Human)

Toledo M, Batista-Gonzalez A, Merheb E et al. Autophagy Regulates the Liver Clock and Glucose Metabolism by Degrading CRY2 Cell Metab. 2018-08-07 [PMID: 29937374] (Mouse)

Rattanaornsompong K, Ngamkham J, Chavalit T, Jitrapakdee S. Generation of Human Pyruvate Carboxylase Knockout Cell Lines Using Retrovirus Expressing Short Hairpin RNA and CRISPR-Cas9 as Models to Study Its Metabolic Role in Cancer Research Methods Mol. Biol. 2019-12-12 [PMID: 30535704] (IF/IHC, Human)

More publications at <http://www.novusbio.com/NBP1-49536>

Procedures

Western Blot protocol for Pyruvate Carboxylase Antibody (NBP1-49536)

Western Blot Protocol

1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH₂O and then 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% NFDM + 1% BSA in TBS + Tween, 1 hour at RT.
6. Rinse the membrane in dH₂O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-Pyruvate Carboxylase primary antibody (NBP1-49536) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH₂O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 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 (Pierce ECL).

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

Immunohistochemistry-Paraffin protocol for Pyruvate Carboxylase Antibody (NBP1-49536)

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 dH₂O three times for 5 minutes each.
2. Wash section in wash buffer (1X PBS/0.1% Tween-20 (1X PBST)) for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1X PBST, 5% goat serum) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul primary antibody diluted in 1X PBST, 5% goat serum to each section. Incubate overnight at 4C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated secondary antibody, diluted in 1X PBST, 5% goat serum. 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 dH₂O.
12. Counterstain sections in hematoxylin.
13. Wash sections in dH₂O two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Immunocytochemistry/Immunofluorescence protocol for Pyruvate Carboxylase Antibody (NBP1-49536)

Immunocytochemistry Protocol

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

1. Pull off culture medium with and add 10% formalin to the dish. Fix at room temperature for 30 minutes..
2. Take off the formalin and add ice cold methanol (kept in well sealed bottle in -20C). Incubate for 5-10 minutes.
3. Take off methanol and add PBS (You can add 0.1% Tween-20 to PBS used here and all subsequent steps), be sure to not let the specimen dry out. Wash 3 times 10 minutes before proceeding to blocking step.
4. To block nonspecific antibody binding incubate in 10% normal goat serum for a minimum of 1 hr at room temp. Cells can also block overnight at 4C for this step.
5. Add primary antibody at appropriate dilution and incubate at room temp for 2 hrs or overnight at room temp.
6. Remove primary antibody and replace with PBS. Wash 3 x 10 min in PBS.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hr at room temperature
8. Remove antibody and replace with PBS, wash 1 x 10 min in PBS. Add Hoechst 33258 to PBS at 1:25,000 and incubate for 10 min. Wash a third time with PBS for 10 min (total of 3X10min PBS washes).
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide and parafilm. Cells can also be coverslipped using Fluoromount. If storing coverslip be sure to seal the edges with clear nail polish.

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





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Products Related to NBP1-49536

NB820-59662	Mouse Liver Whole Tissue Lysate (Adult Whole Normal)
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
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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