# **Product Datasheet**

# PGC1 alpha Antibody - BSA Free NBP1-04676

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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## NBP1-04676

PGC1 alpha 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	91 kDa
Product Description	
Host	Rabbit
Gene ID	10891
Gene Symbol	PPARGC1A
Species	Human, Mouse, Rat, Porcine, Goat, Hamster, Sheep, Squirrel
Reactivity Notes	Use in Rat reported in scientific literature (PMID:35174626, 34573421). Use in Mouse reported in scientific literature (PMID:33719499). Use in Sheep reported in scientific literature (PMID:32403966). Expected from sequence similarity: Mouse
Immunogen	This PGC1 alpha Antibody was developed against a recombinant protein made to an internal portion of the human PGC-1 alpha protein (within residues 400-550). [Swiss-Prot# Q9UBK2].
Product Application Details	
Applications	Western Blot, Simple Western, Chromatin Immunoprecipitation, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry- Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1 - 2 ug/ml, Simple Western 1:25 - 1:80, Chromatin Immunoprecipitation reported by customer review, Flow Cytometry 1 - 2.5 ug/ml, Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence 1:1000. Use reported in scientific literature (PMID 24508229), Immunoprecipitation reported in scientific literature (PMID 24769256), Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen reported in scientific literature (PMID 25981953), Flow (Intracellular) 1 - 2.5 ug/ml, Chromatin Immunoprecipitation (ChIP), Knockout Validated, Knockdown Validated reported in scientific literature (PMID 35455432)
Application Notes	In IHC-P, staining is very strong in the nucleus with some cytoplasmic staining. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.











Analysis of PGC1 alpha Antibody in mouse prostate using DAB with hematoxylin counterstain.



An intracellular stain was performed on HeLa cells with NBP1-04676AF647 (blue) and a matched isotype control (orange). Cells were 400 fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at 300 Relative Cell Number room temperature. Both antibodies were conjugated to Alexa Fluor 647. 200 100 10<sup>5</sup> 10<sup>6</sup> 104 10 0 POC1 alpha Alexa Fluor 647 An intracellular stain was performed on HepG2 cells with PGC1 alpha 400 Antibody and a matched isotype control. Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an 300 antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, Relative Cell Number followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody. 200 100 0 106 104 105 0 PGC1 alpha Copyright @ 2018 Novus Biologicais Western blot shows lysates of A431 human squamous carcinoma 9 parental cell line and PGC1 alpha knockout (KO) A431 cell line. PVDF 4431 PGC1a kD<sub>2</sub> membrane was probed with 1:1000 of Rabbit Polyclonal PGC1 alpha 250 Antibody (Catalog # NBP1-04676) followed by HRP-conjugated Anti-150 Rabbit IgG Secondary Antibody (Catalog #HAF008). Specific band was PGC1-alpha 100 detected for PGC1 alpha at approximately 105 kDa (as indicated) in the 75 parental A431 cell line, but is not detectable in the knockout A431 cell line. This experiment was conducted under reducing conditions. 37 25 20 15 10 Copyright © 2019 Novus Biological GAPDH







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Western Blot: PGC1 alpha Antibody - BSA Free [NBP1-04676] -Maternal exercise during pregnancy on mitochondrial biogenesis in the fetal hearts. (A) Levels of relative mRNA expression measured by  $qRT\_PCR. n = 9-12/group.$  Maternal exercise during pregnancy did not alter levels of mRNA in Ppargc1a & Tfam, while it significantly upregulated the levels of mRNA in Nrf1 & Nrf2. (B–D) Densitometric analyses of protein expression levels relative to the sedentary group with representative images of western blots were shown. No significant differences in PGC $\_1\alpha$ , NRF1, & NRF2 (P > 0.05). n = 5-6/group. \* P < 0.05, significantly different from the sedentary group. Black bar: fetal hearts from sedentary dams; gray bar: fetal hearts from exercised dams. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28292876), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Immunocytochemistry/ Immunofluorescence: PGC1 alpha Antibody -BSA Free [NBP1-04676] - DM1-derived fibroblasts have no changes in mitochondria biogenesis. (A, B) Representative images of immunofluorescence of TOMM20, & PGC1- $\alpha$  in DM1 & control fibroblasts (n=3). (C) Medium fluorescence intensity of MitoTracker Red FM in control (n=3) & DM1 cells (n=5) & (D) of Rhodamine 123 in DM1 & control fibroblasts (n=3). (E) mRNA levels of TFAM transcription factor (n=3). (F) mRNA levels of OPA1, MFN1, MFN2, DRP1 & PARKIN in DM1 & control fibroblasts (n≥2). Image collected & cropped by CiteAb from the following publication

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Western Blot: PGC1 alpha Antibody - BSA Free [NBP1-04676] - Effect of BGP-15 treatment on the regulation of mitochondrial biogenesis in NRCM cells. Western blot analysis of PGC-1- $\alpha$ , CREB, & VDAC proteins as well as densitometric evaluation is shown. GAPDH was used as a loading control. Control group: cells without any treatment; BGP-15 group: cells with only 50  $\mu$ M BGP-15 for 0.5 hours; H2O2 group: cells with 150  $\mu$ M H2O2 for 0.5 hours; H2O2+BGP-15 group: cells with 150  $\mu$ M H2O2 & 50  $\mu$ M BGP-15 for 0.5 hours. Values are mean ± SEM (n = 4).  $\Box p < 0.05$  vs. Control,  $\Box \Box p < 0.01$  vs. Control, §§p < 0.01 vs H2O2 group. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33728024), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



(37 kDa)







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BSA Free [NBP1-04676] - Role of PGC1α in NIX-dependent NT siRNA mitophagy.a-e Nontargeting (NT) or GR siRNA was transfected to hippocampal neurons & SH-SY5Y cells for 24 h prior to corticosterone & cortisol for 12 h, respectively. a, b Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1a) expression was detected Cortisol + NT siRNA in western blot where  $\beta$ -actin was used as a loading control in both cell types. n = 5. c Colocalization of PGC1 $\alpha$  (red) & DAPI (blue) in hippocampal neurons was visualized with SRRF imaging system. Scale bars, 20  $\mu$ m (magnification, ×1000). n = 5. d Colocalization of PGC1 $\alpha$ Cortisol + GR siRNA (green) & DAPI (blue) in SH-SY5Y was visualized with SRRF imaging system. Scale bars, 20 µm (magnification, ×1000). n = 5. e PGC1a protein expressions in subcellular fraction samples were detected by western blotting. Lamin A/C & a-tubulin were used as a nuclear & GR siRNA cytosolic loading control, respectively. n = 5. f, g SH-SY5Y cells were transfected with pcDNA3.1/c-eGFP or pcDNA3.1/PPARGC1A-c-eGFP vector for 24 h prior to cortisol treatment for 24 h. f NIX expression was detected in western blot where  $\beta$ -actin was used as a loading control. n = 5. g TOMM20 levels were detected by western blot. Loading control for western blot is  $\beta$ -actin. n = 5. All blots & immunofluorescence images are representative. n = 5 from independent experiments with two technical replicates each. Quantitative data are presented as a mean ± S.E.M. Two-sided two-way ANOVA was conducted. \*\* indicates p < 0.01 versus control. #, ## indicates p < 0.05, p < 0.01 versus corticosterone in hippocampal neurons & cortisol in SH-SY5Y, respectively. Data are provided as a Source data file. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33473105), licensed under a CC-BY license. Not internally tested by Novus Biologicals. Western Blot: PGC1 alpha Antibody - BSA Free [NBP1-04676] - Role of а PGC1α in NIX-dependent mitophagy.a–e Nontargeting (NT) or GR NT siRNA siRNA was transfected to hippocampal neurons & SH-SY5Y cells for 24 h prior to corticosterone & cortisol for 12 h, respectively. a, b Peroxisome Corticosterone proliferator-activated receptor gamma coactivator 1-alpha (PGC1a) **GR** siRNA expression was detected in western blot where  $\beta$ -actin was used as a loading control in both cell types. n = 5. c Colocalization of PGC1a PGC1a (red) & DAPI (blue) in hippocampal neurons was visualized with SRRF β-actin imaging system. Scale bars, 20 µm (magnification, ×1000). n = 5. d Colocalization of PGC1a (green) & DAPI (blue) in SH-SY5Y was visualized with SRRF imaging system. Scale bars, 20 µm (magnification,  $\times$ 1000). n = 5. e PGC1 $\alpha$  protein expressions in subcellular fraction samples were detected by western blotting. Lamin A/C & α-tubulin were used as a nuclear & cytosolic loading control, respectively. n = 5. f, g SH-SY5Y cells were transfected with pcDNA3.1/c-eGFP or pcDNA3.1/PPARGC1A-c-eGFP vector for 24 h prior to cortisol treatment for 24 h. f NIX expression was detected in western blot where β-actin was used as a loading control. n = 5. g TOMM20 levels were detected by western blot. Loading control for western blot is  $\beta$ -actin. n = 5. All blots & immunofluorescence images are representative. n = 5 from independent experiments with two technical replicates each. Quantitative data are presented as a mean ± S.E.M. Two-sided two-way ANOVA was conducted. \*\* indicates p < 0.01 versus control. #, ## indicates p < 0.05, p < 0.01 versus corticosterone in hippocampal neurons & cortisol in SH-SY5Y, respectively. Data are provided as a Source data file. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33473105), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Immunocytochemistry/ Immunofluorescence: PGC1 alpha Antibody -

105 kDa

43 kDa

d



Western Blot: PGC1 alpha Antibody - BSA Free [NBP1-04676] - EMPA treatment increased mitochondrial biogenesis. (A) Left, Transmission electron microscopy showing the morphology of mitochondria in sham, sham + EMPA, TAC & TAC + EMPA groups & Right, quantitative analysis of mitochondrial size & counts. Results are expressed as mean  $\pm$  SEM, n = 3–5, \*p < 0.05 vs. corresponding sham,  $\pm$ p < 0.05 vs. corresponding TAC. (B) Left, Representative blots of mitochondrial biogenesis-related proteins & Right, quantitative results. (C) Relative mRNA levels of PGC1a, NRF1, TFAM & COX1. Results are expressed as mean  $\pm$  SEM, n = 5–7, \*p < 0.05 vs. corresponding sham group,  $\pm$ p < 0.05 vs. corresponding TAC vehicle group. One-way ANOVA & Tukey post hoc test. EMPA, empagliflozin; SEM, standard error of the mean; TAC, transverse aortic constriction; PGC1- $\alpha$ , peroxisome proliferatoractivated receptor gamma coactivator 1-alpha; NRF-1, nuclear respiratory factor 1; TFAM, mitochondrial transcription factor A; COX1, cyclooxygenase1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35647080), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Immunocytochemistry/ Immunofluorescence: PGC1 alpha Antibody -BSA Free [NBP1-04676] - Role of PGC1α in NIX-dependent mitophagy.a-e Nontargeting (NT) or GR siRNA was transfected to hippocampal neurons & SH-SY5Y cells for 24 h prior to corticosterone & cortisol for 12 h, respectively. a, b Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1a) expression was detected in western blot where  $\beta$ -actin was used as a loading control in both cell types. n = 5. c Colocalization of PGC1 $\alpha$  (red) & DAPI (blue) in hippocampal neurons was visualized with SRRF imaging system. Scale bars, 20  $\mu$ m (magnification, ×1000). n = 5. d Colocalization of PGC1 $\alpha$ (green) & DAPI (blue) in SH-SY5Y was visualized with SRRF imaging system. Scale bars, 20  $\mu$ m (magnification, ×1000). n = 5. e PGC1a protein expressions in subcellular fraction samples were detected by western blotting. Lamin A/C & α-tubulin were used as a nuclear & cytosolic loading control, respectively. n = 5. f, g SH-SY5Y cells were transfected with pcDNA3.1/c-eGFP or pcDNA3.1/PPARGC1A-c-eGFP vector for 24 h prior to cortisol treatment for 24 h. f NIX expression was detected in western blot where  $\beta$ -actin was used as a loading control. n = 5. g TOMM20 levels were detected by western blot. Loading control for western blot is  $\beta$ -actin. n = 5. All blots & immunofluorescence images are representative. n = 5 from independent experiments with two technical replicates each. Quantitative data are presented as a mean ± S.E.M. Two-sided two-way ANOVA was conducted. \*\* indicates p < 0.01 versus control. #, ## indicates p < 0.05, p < 0.01 versus corticosterone in hippocampal neurons & cortisol in SH-SY5Y, respectively. Data are provided as a Source data file. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33473105), licensed under a CC-BY

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Western Blot: PGC1 alpha Antibody - BSA Free [NBP1-04676] -Mitochondrial content & PGC  $\Box$  1 $\alpha$  levels are increased in cells with greater TFEB protein expression. (a) Mitochondrial content was measured in SH SY5Y cells & TFEB DDK cells under basal conditions by assaying citrate synthase (CS) activity. \*\*p < 0.01 versus SH□SY5Y; n = 6. (b) Western blotting for the mitochondrial proteins prohibitin 1 &

SY5Y cells were transfected with pcDNA3.1/c-eGFP or pcDNA3.1/PPARGC1A-c-eGFP vector for 24 h prior to cortisol treatment for 24 h. f NIX expression was detected in western blot where β-actin was used as a loading control. n = 5. g TOMM20 levels were detected by western blot. Loading control for western blot is  $\beta$ -actin. n = 5. All blots & immunofluorescence images are representative. n = 5 from independent experiments with two technical replicates each. Quantitative data are presented as a mean ± S.E.M. Two-sided two-way ANOVA was conducted. \*\* indicates p < 0.01 versus control. #, ## indicates p < 0.05, p < 0.01 versus corticosterone in hippocampal neurons & cortisol in SH-SY5Y, respectively. Data are provided as a Source data file. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33473105), licensed under a CC-BY

PGC1α in NIX-dependent mitophagy.a–e Nontargeting (NT) or GR

proliferator-activated receptor gamma coactivator 1-alpha (PGC1α)

expression was detected in western blot where  $\beta$ -actin was used as a loading control in both cell types. n = 5. c Colocalization of PGC1 $\alpha$ 

Colocalization of PGC1α (green) & DAPI (blue) in SH-SY5Y was

×1000). n = 5. e PGC1α protein expressions in subcellular fraction

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(red) & DAPI (blue) in hippocampal neurons was visualized with SRRF imaging system. Scale bars, 20  $\mu$ m (magnification, ×1000). n = 5. d

cytochrome oxidase (COX) subunit IV also indicated that mitochondrial content was increased in TFEB DDK cells. (c) PGC 1 $\alpha$  mRNA levels were measured in SHOSY5Y cells & TFEBODDK cells under basal conditions by qPCR. Data normalised against  $\beta$  actin mRNA levels. \*\*p < 0.01 versus SH SY5Y cells; n = 3. (d) SH SY5Y cells were treated with scrambled (scram) or TFEB siRNA for 72 h & PGC □ 1α mRNA levels measured. \*p < 0.05 versus scram; n = 4. (e) PGC  $\Box$  1a protein levels were detected by western blotting in total cell lysates of SH SY5Y cells, TFEB DDK cells & a SH SY5Y cell line over expressing human PGC 1 $\alpha$ . The fold increase in PGC 1 $\alpha$  protein density for this blot & PGC  $\square$  1 $\alpha$  mRNA levels of the respective cell lines are reported underneath the blot. (f) TFEB DDK cells were treated with 10 µM carbonyl cyanide m chlorophenylhydrazone (CCCP) for 18 h & cytosolic & nuclear fractions prepared. PGC 1α protein was detected in cytosolic (3.5% of total volume) & nuclear fractions (35% of total volume) by western blotting. The purity of the nuclear & cytosolic fractions was assessed by western blotting using lamin A & β actin antibodies respectively. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26509433), licensed under a CC-BY license. Not internally tested by Novus Biologicals.







Western Blot: PGC1 alpha Antibody - BSA Free [NBP1-04676] - Effects SDHb optic nerve of SRTAW04 on expression of markers of mitochondrial & anti-oxidant SOD2 optic nerve B-actin optic nerve function. (a) Western blot of protein extracts from optic nerve & retina of SDHb retina control (lanes 1-4), MHV-A59 infected (lanes 5-8), & MHV-A59 infected SOD2 retina + SRTAW04-treated (lanes 9–12) mice. Average levels of SDHb B-actin retina measured by Western blotting (n = 4/group) showed a significant (\*p < PGC1q retina B-actin retina 0.05) decrease in protein extracts from optic nerves (a,b) & retinas (a,d) of MHV-A59 infected mice 7 days post-inoculation, compared to control mice. MHV-A59 infected mice treated with SRTAW04 (100 mg/kg/day) showed a significant increase (@p < 0.05) of SDHb protein expression compared to untreated MHV-A59 infected mice. There is a significant decrease (\*p < 0.05) in expression of SOD2 (n = 4/group) in optic nerves (a,c) & retinas (a,e) of MHV-A59 infected mice compared to control mice, & treatment with SRTAW04 significantly (@p < 0.05) attenuates that change. PGC1- $\alpha$  expression shows a significant (\*p < 0.05) decrease in retinas (a,f) (n = 4/group) during MHV-A59 infection & treatment with SRTAW04 for 7 days significantly (@p < 0.05) increases the PGC1- $\alpha$ protein levels. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24383546), licensed under a CC-BY license. Not internally tested by Novus Biologicals. An intracellular stain was performed on A431 human skin carcinoma cell line using Rabbit anti-PGC1 alpha Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP1-04676AF647, blue Relative Cell Number histogram) or matched control antibody (Catalog # NBP2-24981AF647, orange histogram) at 2.5 µg/mL for 30 minutes at RT. 200



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#### **Publications**

Miao ZF, Sun JX, Huang XZ, Bai S et Al. Metaplastic regeneration in the mouse stomach requires a reactive oxygen species pathway Dev Cell 2024-03-23 [PMID: 38521055]

Kopsidas CA, Lowe CC, McDaniel DP, Zhou X et AI. Sustained generation of neurons destined for neocortex with oxidative metabolic upregulation upon filamin abrogation iScience 2024-07-11 [PMID: 38989458]

Torres JA, Holznecht N, Asplund DA, Kroes BC et Al. ?-hydroxybutyrate recapitulates the beneficial effects of ketogenic metabolic therapy in polycystic kidney disease iScience 2024-09-24 [PMID: 39314240]

Rosenkranz SC, Shaposhnykov AA, Träger S, Engler JB et AI. Enhancing mitochondrial activity in neurons protects against neurodegeneration in a mouse model of multiple sclerosis Elife 2021-02-10 [PMID: 33565962]

Wen X, Song Y, Zhang M et Al. Polyphenol Compound 18a Modulates UCP1-Dependent Thermogenesis to Counteract Obesity Biomolecules 2024-05-23 [PMID: 38927022]

Ren D, He Z, Fedorova J et Al. Sestrin2 maintains OXPHOS integrity to modulate cardiac substrate metabolism during ischemia and reperfusion Redox Biol 2020-12-01 [PMID: 33316744]

Samta V, Fan T, Youssef M et al. A transcriptional regulatory mechanism of genes in the tricarboxylic acid cycle in the heart J Biol Chem. 2024-08-14 [PMID: 39151728]

Liu C, Fu Z, Wu S et Al. Mitochondrial HSF1 triggers mitochondrial dysfunction and neurodegeneration in Huntington's disease EMBO Mol Med 2022-06-07 [PMID: 35670111]

Zheng Y, Wang Y, Xiong X et Al. CD9 Counteracts Liver Steatosis and Mediates GCGR Agonist Hepatic Effects Adv Sci (Weinh) 2024-06-05 [PMID: 38837628]

Wu D, Yang Y, Hou Y et Al. Increased mitochondrial fission drives the reprogramming of fatty acid metabolism in hepatocellular carcinoma cells through suppression of Sirtuin 1 Cancer Commun (Lond) 2022-01-04 [PMID: 34981667] (Immunohistochemistry, Western Blot)

Torres JA, Holznecht N, Asplund DA et Al. A combination of ?-hydroxybutyrate and citrate ameliorates disease progression in a rat model of polycystic kidney disease Am J Physiol Renal Physiol 2024-03-01 [PMID: 38095025]

Hombrebueno JR, Cairns L, Dutton LR et Al. Uncoupled turnover disrupts mitochondrial quality control in diabetic retinopathy JCI Insight 2019-12-05 [PMID: 31661466] (Immunohistochemistry, Western Blot)

More publications at http://www.novusbio.com/NBP1-04676



#### **Procedures**

#### Western Blot protocol for PGC1 alpha Antibody (NBP1-04676)

#### Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.

2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.

3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.

4. Rinse the blot.

5. Block the membrane using standard blocking buffer for at least 1 hour.

6. Wash the membrane in wash buffer three times for 10 minutes each.

7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.

8. Wash the membrane in wash buffer three times for 10 minutes each.

9. Apply the diluted 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 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.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

#### Immunohistochemistry-Paraffin Protocol for PGC1 alpha Antibody (NBP1-04676)

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.

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



Immunocytochemistry/Immunofluorescence Protocol for PGC1 alpha Antibody (NBP1-04676) 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 NBP1-04676

NB820-59668	Mouse Skeletal Muscle Whole Tissue Lysate (Adult Whole Normal)
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/NBP1-04676

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