Product Datasheet

PGAM1/2/4 Antibody
NB100-774

Unit Size: 0.1 mg

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

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Publications: 5

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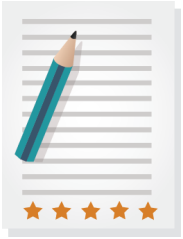
Updated 10/23/2024 v.20.1



NB100-774

PGAM1/2/4 Antibody

Product Information

Unit Size	0.1 mg
Concentration	0.5 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris saline (20 mM Tris pH 7.3, 150 mM NaCl), 0.5% BSA
Target Molecular Weight	29 kDa

Product Description

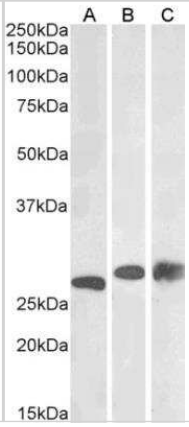
Host	Goat
Gene ID	5223
Gene Symbol	PGAM1
Species	Human, Mouse, Rat, Porcine
Specificity/Sensitivity	Please note this antibody is expected to recognize the products of 3 highly similar genes.
Immunogen	Peptide with sequence C-KAMEAVAAQGKAKK, from the C Terminus of the protein sequence according to NP_002620.1; NP_000281.2; NP_001025062.1.

Product Application Details

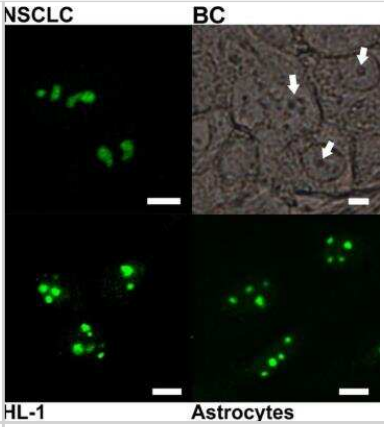
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Peptide ELISA
Recommended Dilutions	Western Blot 0.05-0.15 ug/ml, Immunocytochemistry/ Immunofluorescence, Peptide ELISA Detection limit 1:28000
Application Notes	WB: Approx 27kDa band observed in Human Cerebellum and in Human and Mouse Liver lysates, and approx. 28kDa band observed in Rat and Pig Liver lysates (calculated MW of 28.8kDa according to Human NP_002620.1, NP_000281.2, and NP_001025062.1, Mouse NP_075907.2, Rat NP_445742.2 and Pig XP_003483583.1).

Images

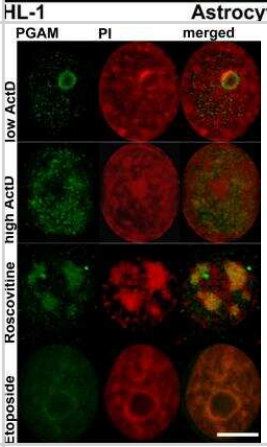
Western Blot: PGAM1/2/4 Antibody [NB100-774] - staining of Mouse (A), Rat (B) and Pig (C) Liver lysate (35ug protein in RIPA buffer). Primary incubation was 1 hour. Detected by chemiluminescence.



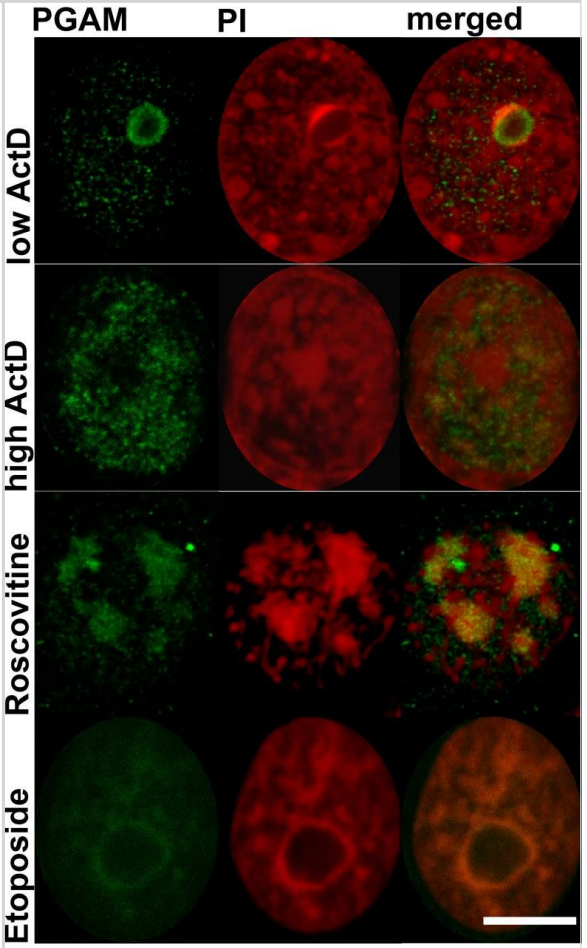
Immunocytochemistry/Immunofluorescence: PGAM1/2/4 Antibody [NB100-774] - Subcellular localization of PGAM with the use of antibody directed to C-terminal peptide of the proteinThe localization was examined in cultures of non-small cell lung carcinoma (NSCLC) HL-1 cardiomyocytes, astrocytes and in breast cancer tissue section (BC). Arrows point nucleoli. Bar=5 um. Image collected and cropped by CiteAb from the following publication (oncotarget.com/lookup/doi/10.18632/oncotarget.4044), licensed under a CC-BY license.

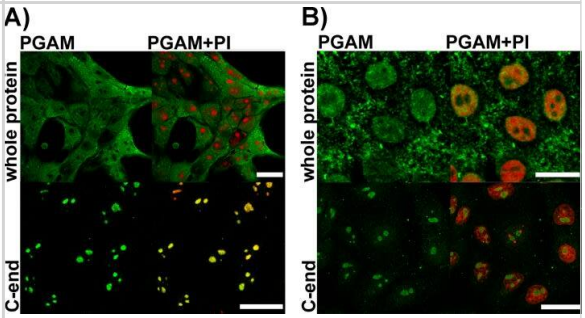


Immunocytochemistry/Immunofluorescence: PGAM1/2/4 Antibody [NB100-774] - Localization of PGAM with antibody directed to the C-terminal peptide of the protein -in nuclei of KLN-205 cells treated with drugs disturbing ribosomal biogenesisActD = actinomycin D; PI - propidium iodide. Bar=5 um. Image collected and cropped by CiteAb from the following publication (oncotarget.com/lookup/doi/10.18632/oncotarget.4044), licensed under a CC-BY license.



Validation of R-Ras2 as the defatty-acylation target of SIRT6.(A) mRNA and protein levels of R-Ras2 in Sirt6 WT and KO MEFs. (B) In-gel fluorescence (with NH2OH treatment) showing that R-Ras2 has higher lysine fatty acylation level in Sirt6 KO MEFs than in Sirt6 WT MEFs. Right histogram shows the quantification of bands on the fluorescence gel. Values with error bars indicate mean ± s.d. of three biological replicates. * indicates p<0.05. The full fluorescence gel is shown in Figure 2—figure supplement 2A. (C) Detection of R-Ras2 lysine fatty acylation levels in control and SIRT6 knockown HEK 293 T cells by in-gel fluorescence. Right histogram shows the quantification of bands on the fluorescence gel. Values with error bars indicate mean ± s.d. of three biological replicates. * indicates p<0.05. (D) Lysine fatty acylation levels of endogenous R-Ras2 in Sirt6 WT and KO MEFs. (E, F) SIRT6 defatty-acylated R-Ras2 in a NAD+-dependent manner in vitro. In-gel fluorescence was used to detect R-Ras2 lysine fatty acylation (E). A 32P-NAD+ assay was used to detect fatty acyl ADPR product from defatty-acylation reaction. (F). (G) In-gel fluorescence (with NH2OH treatment) showing that mutation of four lysine residues at the C-terminus of R-Ras2 significantly decreased lysine fatty acylation in Sirt6 KO MEFs. Right histogram shows the quantification of bands on the fluorescence gel. Values with error bars indicate mean ± s.d. of three biological replicates. * indicates p<0.05. The full fluorescence gel including R-Ras2 total fatty acylation levels (without NH2OH treatment) is shown in Figure 2—figure supplement 2C. (H) Tandem mass (MS/MS) spectrum of doubly charged Alk14 modified (on K194) R-Ras2 peptide. The b- and y- ions are shown along with the peptide sequence. (I) In-gel fluorescence (with NH2OH treatment) showing that single mutation of four lysine residues at the C-terminus of R-Ras2 did not affect R-Ras2 lysine fatty acylation. (J) Confocal imaging showed that R-Ras2 WT was mainly localized in the plasma membrane in Sirt6 KO MEFs. R-Ras2 WT in Sirt6 WT MEFs as well as R-Ras2 4KR in Sirt6 WT and KO MEFs was localized in both the intracellular vesicles and plasma membrane (n = 5, 5, 5, 6 cells for each sample from left to right, respectively. The images



of other cells were shown in Figure 2—figure supplement 4A.DOI:https://dx.doi.org/10.7554/eLife.25158.005Scheme showing the in-gel fluorescence method with Alk14 metabolic labeling to identify R-Ras2 as a lysine fatty acylated protein.FLAG-tagged R-Ras2 protein was enriched from whole cell lysates by FlAG immunoprecipitation. Alk14-labeled R-Ras2 protein was detected by in-gel fluorescence after incorporating BODIPY (B)-azide using click chemistry.DOI:https://dx.doi.org/10.7554/eLife.25158.006Validation of R-Ras2 as the defatty-acylation target of SIRT6. (A) Full gel image of R-Ras2 fatty acylation level with or without NH2OH treatment in Sirt6 WT and KO MEFs. (B) Lysine fatty acylation levels of overexpressed R-Ras2 in Sirt6 WT and KO MEFs. (C) Full gel image of R-Ras2 WT and 4KR fatty acylation levels with or without NH2OH treatment in Sirt6 KO MEFs. (D) In-gel fluorescence showing lysine fatty acylation level of overexpressed R-Ras2 WT and C199S mutant in HEK 293T cells with SIRT6 knockdown. C, control shRNA. S6, SIRT6 shRNA#1.DOI:https://dx.doi.org/10.7554/eLife.25158.007Total ion chromatogram (TIC), extracted ion chromatogram (XIC) and parent MS (MS1) of Ak14 modified R-Ras2 peptide.DOI:https://dx.doi.org/10.7554/eLife.25158.008Lysine fatty acylation targets R-Ras2 to plasma membrane.(A) Confocal imaging showing subcellular localization of GFP-tagged R-Ras2 WT and 4KR in Sirt6 WT and KO MEFs. (B) Subcellular fractionation of R-Ras2 WT and 4KR in HEK 293T cells with palmitic acid or TM3 treatment. GAPDH was used as the marker of cytosol fraction and Na,K-APTase was used as the marker of plasma membrane fraction.DOI:https://dx.doi.org/10.7554/eLife.25158.009 Image collected and cropped by CiteAb from the following open publication (https://elifesciences.org/articles/25158), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunocytochemistry/ Immunofluorescence: PGAM1/2/4 Antibody [NB100-774] - Detection of PGAM in KLN-205 cells with antibodies directed to whole PGAM protein or to C-terminal peptide of PGAMA. control conditions (scan parameters in red channel were set to emphasize nucleolar staining with propidium iodide – PI) B. RNase-treated cells. Bar=15 µm. Image collected & cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.4044), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Ritu Chaudhary, Robbert J C Slebos, Feifei Song, Keegan P McCleary-Sharpe, Jude Masannat, Aik Choon Tan, Xuefeng Wang, Nelusha Amaladas, Wenjuan Wu, Gerald E Hall, Jose R Conejo-Garcia, Juan C Hernandez-Prera, Christine H Chung Effects of checkpoint kinase 1 inhibition by prexasertib on the tumor immune microenvironment of head and neck squamous cell carcinoma. Molecular carcinogenesis 2021-03-22 [PMID: 33378592]

Gizak A, McCubrey JA, Rakus D. Cell-to-cell lactate shuttle operates in heart and is important in age-related heart failure Aging (Albany NY) 2020-02-08 [PMID: 32035422]

Gizak A, Grenda M, Mamczur P et al. Insulin/IGF1-PI3K-dependent nucleolar localization of a glycolytic enzyme--phosphoglycerate mutase 2, is necessary for proper structure of nucleolus and RNA synthesis. Oncotarget 2015-07-10 [PMID: 26033454]

Sakoda S, Shanske S, DiMauro S, Schon EA. Isolation of a cDNA encoding the B isozyme of human phosphoglycerate mutase (PGAM) and characterization of the PGAM gene family. J Biol Chem 1988-11-15 [PMID: 2846553]

Chaneton B, Hillmann P, Zheng L et al. Serine is a natural ligand and allosteric activator of pyruvate kinase M2 Nature 2012-10-14 [PMID: 23064226] (Human)

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