

# Product Datasheet

## PKM1 Antibody - BSA Free

### NBP2-14833

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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**NBP2-14833**

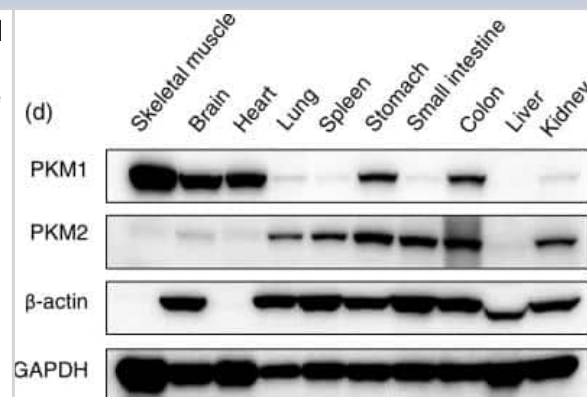
PKM1 Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.17 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	Immunogen affinity purified
Buffer	PBS and 30% Glycerol
Product Description	
Host	Rabbit
Gene ID	5315
Gene Symbol	PKM
Species	Human, Mouse, Bovine
Reactivity Notes	Bovine reactivity reported in scientific literature (PMID: 25416385).
Specificity/Sensitivity	This antibody is specific for the M1 isoform of PKM.
Immunogen	A synthetic peptide made to a C-terminal portion of the human PKM1 protein (between residues 350-450) [UniProt P14618-2].
Product Application Details	
Applications	Western Blot, Simple Western, Immunoblotting, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 0.5 ug/ml, Simple Western 1:400, Immunohistochemistry 1:400, Immunocytochemistry/ Immunofluorescence reported in multiple pieces of scientific literature, Immunohistochemistry-Paraffin 1:400, Immunoblotting reported in scientific literature (PMID 25416385)
Application Notes	<p>In Western blot a band is seen ~57 kDa in human and mouse skeletal muscle, brain and heart. In IHC-P, staining was observed in the cytoplasm of human breast cancer tissue. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.</p> <p>In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.</p> <p>See <a href="#">Simple Western Antibody Database</a> for Simple Western validation: Tested in Human Brain lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:400, apparent MW was 59 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.</p>

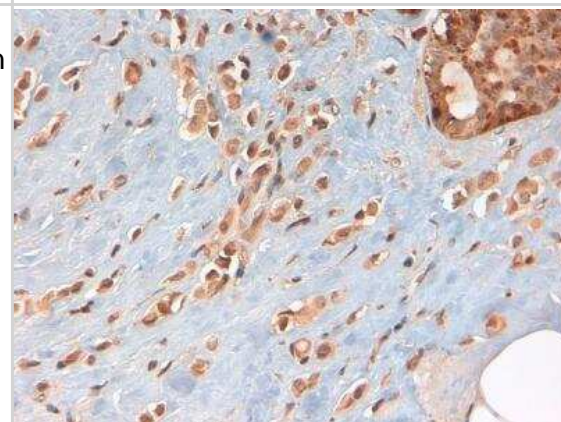


## Images

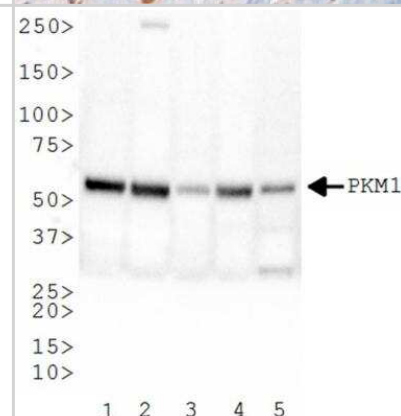
Western Blot: PKM1 Antibody [NBP2-14833] - Expression profile of PKM isoforms in tissues from mouse organs. PKM1 and PKM2 were detected by Western blotting in under the same experimental conditions as Figure 1a at the same time. The full-length blots are presented in Supplementary Figure S2b. Results are presented as the mean  $\pm$  SD (\*\* P < 0.01). Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep08647>), licensed under a CC-BY license.



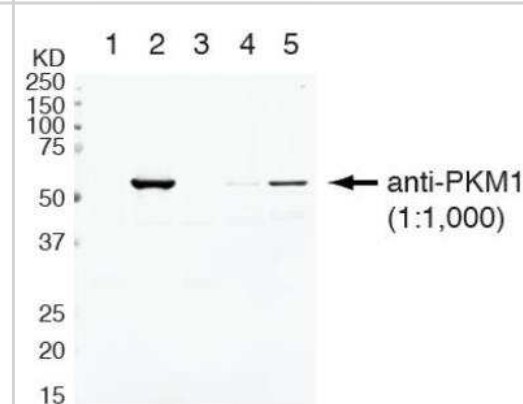
Immunohistochemistry-Paraffin: PKM1 Antibody [NBP2-14833] - PKM1 antibody was tested in human breast cancer using DAB with hematoxylin counterstain.

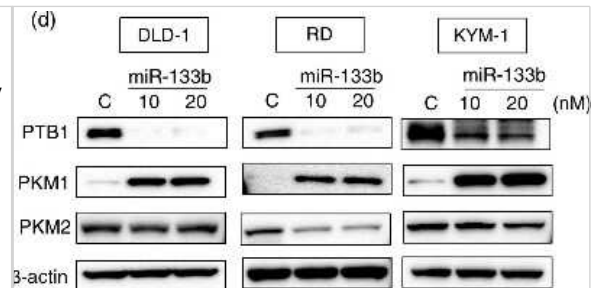


Western Blot: PKM1 Antibody [NBP2-14833] - PKM1 antibody was tested in 1. human skeletal muscle 2. mouse skeletal muscle 3. human brain 4. mouse brain and 5. human heart cell lysate.

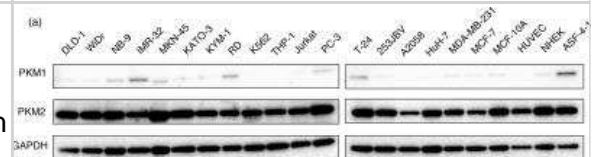


Western Blot: PKM1 Antibody [NBP2-14833] - Detection of PKM1 on HEK293T and U-87 cells. Lane 1: HEK293T cells (major isoform is PKM2, human). Lane 2: HEK293T cells with plasmid overexpressing mouse PKM1. Lane 3: HEK293T cells with plasmid overexpressing mouse PKM2. Lane 4: U-87 cells with control shRNA (major isoform is PKM2, human). Lane 5: U-87 cells with PTB/A1/A2 shRNAs (major isoform is PKM1, human). Photo courtesy of product review by verified customer.

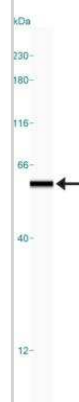




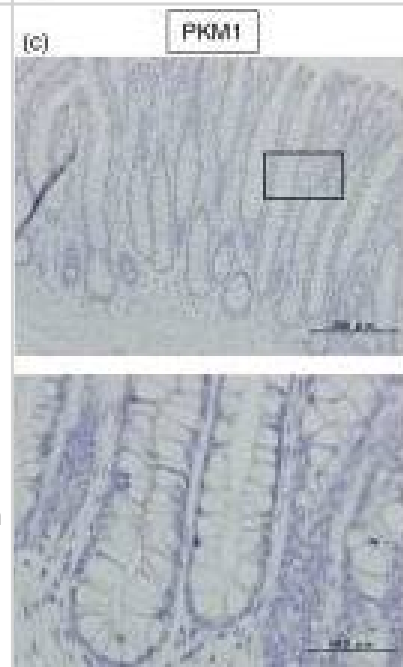
Western Blot: PKM1 Antibody [NBP2-14833] - Expression profile of PKM isoforms in various cancer cell lines, control cell lines, and cells in primary culture. PKM1 and PKM2 were detected by Western blotting under the same experimental conditions at the same time. The full-length blots are presented in Supplementary Figure S2a. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep08647>), licensed under a CC-BY license.



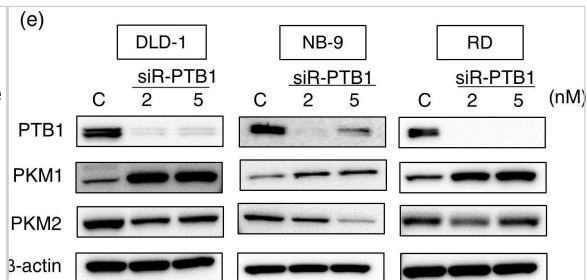
Simple Western: PKM1 Antibody [NBP2-14833] - Simple Western lane view shows a specific band for PKM1 in 0.5 mg/ml of Human Brain lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



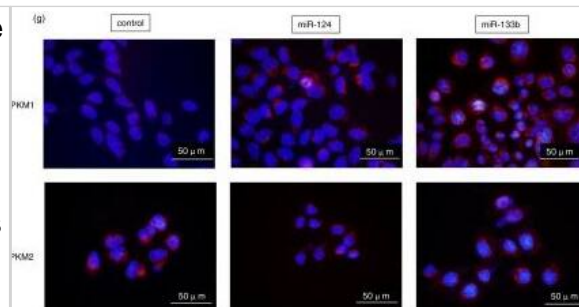
Immunohistochemistry-Paraffin: PKM1 Antibody - BSA Free [NBP2-14833] - Expression of PKM1 & PKM2 in clinical colorectal cancer samples. (a) The protein expression of PKM1 & PKM2 in clinical specimens of cancer tumor (T) & the adjacent normal tissues (N) is shown. PKM1 & PKM2 were detected by Western blotting in under the same experimental conditions at the same time. The full-length blots are presented in Supplementary Figure S3b. (b–d) Immunohistochemical staining of normal colon tissue adjacent to tumor tissue of case 10. Results of H&E staining (b), staining with anti-PKM1 antibody (c), & staining with anti-PKM2 (d) are shown. The boxed regions in “c” & “d” are enlarged in the images below. (e–h) Immunohistochemical staining of clinical colorectal cancer tissue specimen of representative case 3. H&E-stained section with normal tissue (upper right corner) neighboring the tumor area in the section is shown (e), along with the same section stained with anti-PKM2 antibody (f). Enlarged views of boxed areas in “f” show normal colorectal crypt in mucosa (g) & tumor area (h) stained with anti-PKM2 antibody. Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep08647>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: PKM1 Antibody - BSA Free [NBP2-14833] - (a) Luciferase activities after co-transfection of DLD-1 cells w/ control or miR-124 (wild-type or mutant-type) pMIR vectors having predictive miR-124 binding site in 3'UTR of PTB1. Upper panel region of 3'-UTR of human PTB1 mRNA complementary to mature miR-124. Box indicates predicted binding sites for miR-124. (b) Same as "a" except miR-133b used. (c) Expression of PTB1, PKM1, & PKM2 proteins at 72 h after transfection of DLD-1, NB9 or IMR-32 cells w/ miR-124 (10, 20 or 40 nM). (d) Expression of PTB1, PKM1, & PKM2 proteins at 72 h after transfection of DLD-1, RD or KYM-1 cells w/ miR-133b (10, 20 nM). (e) Expression of PTB1, PKM1, & PKM2 proteins at 72 h after transfection of DLD-1, NB-9 or RD cells w/ siR-PTB1 (2, 5 nM). (f) Effect of combined treatment of DLD-1 cells w/ antagomiR-124 & miR-124 or antagomiR-133b & miR-133b. DLD-1 cells transfected w/ non-specific control, miR-124/miR-133b (10 nM), miR-124/miR-133b (10 nM) + antagomiR-124/antagomiR-133b (5 nM) or miR-124/miR-133b (10 nM) + antagomiR-124/antagomiR-133b (10 nM). Expression level of PTB1 assessed at 48 h after transfection. The full-length blots are presented in Supplementary Figure S3a. (g) IF of PKM1 (upper panels) & PKM2 (lower panels) at 48 h after transfection of DLD-1 cells w/ miR-124 (20 nM) or miR-133b (20 nM). Left panels, treatment w/ control miRNA; middle panels, treatment w/ miR-124; right panels, treatment w/ miR-133b. PKM1 or PKM2 is stained red, & nuclei are stained blue. (h) Lactate production measured at 48 h after transfection of DLD-1 cells w/ miR-124 (20 nM), miR-133b (20 nM) or siR-PTB1 (5 nM). Results are presented as mean  $\pm$  SD (\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; N.S., not statistically significant). Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep08647>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

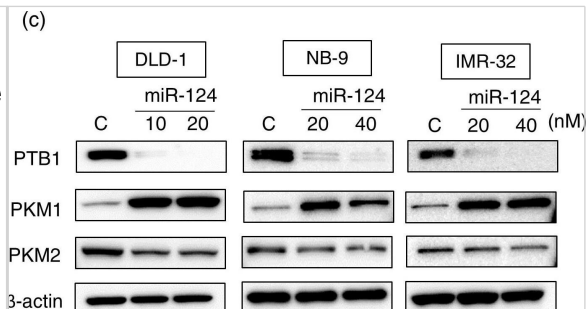


Immunocytochemistry/ Immunofluorescence: PKM1 Antibody - BSA Free [NBP2-14833] - (a) Luciferase activities after co-transfection of DLD-1 cells w/ control or miR-124 (wild-type or mutant-type) pMIR vectors having predictive miR-124 binding site in 3'UTR of PTB1. Upper panel region of 3'-UTR of human PTB1 mRNA complementary to mature miR-124. Box indicates predicted binding sites for miR-124. (b) Same as "a" except miR-133b used. (c) Expression of PTB1, PKM1, & PKM2 proteins at 72 h after transfection of DLD-1, NB9 or IMR-32 cells w/ miR-124 (10, 20 or 40 nM). (d) Expression of PTB1, PKM1, & PKM2 proteins at 72 h after transfection of DLD-1, RD or KYM-1 cells w/ miR-133b (10, 20 nM). (e) Expression of PTB1, PKM1, & PKM2 proteins at 72 h after transfection of DLD-1, NB-9 or RD cells w/ siR-PTB1 (2, 5 nM). (f) Effect of combined treatment of DLD-1 cells w/ antagomiR-124 & miR-124 or antagomiR-133b & miR-133b. DLD-1 cells transfected w/ non-specific control, miR-124/miR-133b (10 nM), miR-124/miR-133b (10 nM) + antagomiR-124/antagomiR-133b (5 nM) or miR-124/miR-133b (10 nM) + antagomiR-124/antagomiR-133b (10 nM). Expression level of PTB1 assessed at 48 h after transfection. The full-length blots are presented in Supplementary Figure S3a. (g) IF of PKM1 (upper panels) & PKM2 (lower panels) at 48 h after transfection of DLD-1 cells w/ miR-124 (20 nM) or miR-133b (20 nM). Left panels, treatment w/ control miRNA; middle panels, treatment w/ miR-124; right panels, treatment w/ miR-133b. PKM1 or PKM2 is stained red, & nuclei are stained blue. (h) Lactate production measured at 48 h after transfection of DLD-1 cells w/ miR-124 (20 nM), miR-133b (20 nM) or siR-PTB1 (5 nM). Results are presented as mean  $\pm$  SD (\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001; N.S., not statistically significant). Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep08647>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

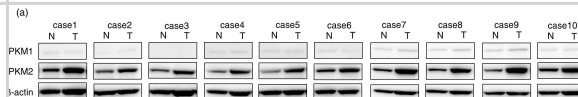




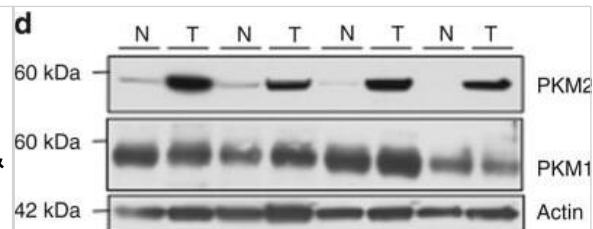
**Western Blot: PKM1 Antibody - BSA Free [NBP2-14833] - (a)** Luciferase activities after co-transfection of DLD-1 cells w/ control or miR-124 (wild-type or mutant-type) pMIR vectors having predictive miR-124 binding site in 3'UTR of PTB1. Upper panel region of 3'-UTR of human PTB1 mRNA complementary to mature miR-124. Box indicates predicted binding sites for miR-124. (b) Same as "a" except miR-133b used. (c) Expression of PTB1, PKM1, & PKM2 proteins at 72 h after transfection of DLD-1, NB9 or IMR-32 cells w/ miR-124 (10, 20 or 40 nM). (d) Expression of PTB1, PKM1, & PKM2 proteins at 72 h after transfection of DLD-1, RD or KYM-1 cells w/ miR-133b (10, 20 nM). (e) Expression of PTB1, PKM1, & PKM2 proteins at 72 h after transfection of DLD-1, NB-9 or RD cells w/ siR-PTB1 (2, 5 nM). (f) Effect of combined treatment of DLD-1 cells w/ antagomiR-124 & miR-124 or antagomiR-133b & miR-133b. DLD-1 cells transfected w/ non-specific control, miR-124/miR-133b (10 nM), miR-124/miR-133b (10 nM) + antagomiR-124/antagomiR-133b (5 nM) or miR-124/miR-133b (10 nM) + antagomiR-124/antagomiR-133b (10 nM). Expression level of PTB1 assessed at 48 h after transfection. The full-length blots are presented in Supplementary Figure S3a. (g) IF of PKM1 (upper panels) & PKM2 (lower panels) at 48 h after transfection of DLD-1 cells w/ miR-124 (20 nM) or miR-133b (20 nM). Left panels, treatment w/ control miRNA; middle panels, treatment w/ miR-124; right panels, treatment w/ miR-133b. PKM1 or PKM2 is stained red, & nuclei are stained blue. (h) Lactate production measured at 48 h after transfection of DLD-1 cells w/ miR-124 (20 nM), miR-133b (20 nM) or siR-PTB1 (5 nM). Results are presented as mean  $\pm$  SD (\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; N.S., not statistically significant). Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep08647>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



**Western Blot: PKM1 Antibody - BSA Free [NBP2-14833] - Expression of PKM1 & PKM2 in clinical colorectal cancer samples.**(a) The protein expression of PKM1 & PKM2 in clinical specimens of cancer tumor (T) & the adjacent normal tissues (N) is shown. PKM1 & PKM2 were detected by Western blotting in under the same experimental conditions at the same time. The full-length blots are presented in Supplementary Figure S3b. (b–d) Immunohistochemical staining of normal colon tissue adjacent to tumor tissue of case 10. Results of H&E staining (b), staining with anti-PKM1 antibody (c), & staining with anti-PKM2 (d) are shown. The boxed regions in "c" & "d" are enlarged in the images below. (e–h) Immunohistochemical staining of clinical colorectal cancer tissue specimen of representative case 3. H&E-stained section with normal tissue (upper right corner) neighboring the tumor area in the section is shown (e), along with the same section stained with anti-PKM2 antibody (f). Enlarged views of boxed areas in "f" show normal colorectal crypt in mucosa (g) & tumor area (h) stained with anti-PKM2 antibody. Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep08647>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



**Western Blot: PKM1 Antibody - BSA Free [NBP2-14833] -**  
**Characterization of NOX4 & PKM2 in human RCC tumors & adjacent tissue.** a Mitochondrial fractions were prepared from human tumors (T) or uninvolved adjacent tissue (N). NOX4 expression was examined by western blot analysis. Prohibitin was probed as a mitochondrial marker & loading control. b Quantitation of NOX4 distribution in the mitochondrial fraction from a. The results are expressed as the means using one-way ANOVA with Tukey's post hoc test where  $\pm$  S.E.M. \* $p < 0.05$  compared to normal (N). c Mitochondria fractions were prepared from RCC tumors & NADPH-dependent superoxide generation was examined in the presence (+) or absence (-) of ATP. The results are from eight tumors & are expressed as the means using one-way ANOVA with Tukey's post hoc test where  $\pm$  S.E.M. \*\* $p < 0.01$  is compared to without (-) ATP. d PKM2 & PKM1 expression was examined by western blot analysis in lysates prepared from human tumors (T) or uninvolved adjacent tissue (N) from the same patient. Actin as loading control Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/29051480>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.





## Publications

Li Y, Chen X, Huang H et al. A feedback loop between NONHSAT024276 and PTBP1 inhibits tumor progression and glycolysis in HCC by increasing the PKM1/PKM2 ratio *Cancer Science* 2023-04-01 [PMID: 36529521] (Western Blot)

Deck M, Van Hameren G, Campbell G et al. Physiology of PNS axons relies on glycolytic metabolism in myelinating Schwann cells *PloS one* 2022-10-04 [PMID: 36194565] (WB, IHC-Fr, Mouse)

Han J, Hyun J, Park J et al. Aberrant role of pyruvate kinase M2 in the regulation of gamma-secretase and memory deficits in Alzheimer's disease *Cell reports* 2021-12-07 [PMID: 34879266]

Tian, L, Xiong, P Y Et al. Supra-coronary aortic banding improves right ventricular function in experimental pulmonary arterial hypertension in rats by increasing systolic right coronary artery perfusion. *Acta Physiol (Oxf)* 2020-08-01 [PMID: 32339403] (ICC/IF, Human)

Zhang H, Wang D et al. Metabolic and Proliferative State of Vascular Adventitial Fibroblasts in Pulmonary Hypertension Is Regulated Through a MicroRNA-124/PTBP1 (Polypyrimidine Tract Binding Protein 1)/Pyruvate Kinase Muscle Axis. *Circulation* 2017-12-19 [PMID: 28972001] (WB, Bovine)

Okazaki Mitsuyoshi, Fushida Sachio, Tsukada Tomoya et al. The effect of HIF-1a and PKM1 expression on acquisition of chemoresistance. *Cancer Management and Research* 2018-01-01 [PMID: 30013393] (WB, IF/IHC, Mouse)

Shanmugasundaram K, Nayak BK, Friedrichs WE et al. NOX4 functions as a mitochondrial energetic sensor coupling cancer metabolic reprogramming to drug resistance. *Nat Commun.* 2017-10-19 [PMID: 29051480] (WB, Human)

Minami K, Taniguchi K, Sugito N et al. MiR-145 negatively regulates Warburg effect by silencing KLF4 and PTBP1 in bladder cancer cells. *Oncotarget.* 2017-05-16 [PMID: 28380435] (WB, Human)

Takai T, Yoshikawa Y, Inamoto T et al. A Novel Combination RNAi toward Warburg Effect by Replacement with miR-145 and Silencing of PTBP1 Induces Apoptotic Cell Death in Bladder Cancer Cells *Int J Mol Sci* 2017-01-17 [PMID: 28106737] (WB, Human)

Sugiyama T, Taniguchi K, Matsushashi N et al. MiR-133b inhibits growth of human gastric cancer cells by silencing pyruvate kinase muscle-splicer polypyrimidine tract-binding protein 1. *Cancer Sci.* 2016-12-01 [PMID: 27696637] (WB, Human)

J Casson R, P M Wood J, Han G et al. M-Type Pyruvate Kinase Isoforms and Lactate Dehydrogenase A in the Mammalian Retina: Metabolic Implications. *Invest. Ophthalmol. Vis. Sci.* 2016-01-01 [PMID: 26780311] (WB, IHC-P, Mouse)

Taniguchi K, Sugito N, Kumazaki M et al. Positive feedback of DDX6/c-Myc/PTB1 regulated by miR-124 contributes to maintenance of the Warburg effect in colon cancer cells *Biochim. Biophys. Acta.* 2015-07-02 [PMID: 26144048] (WB, Human)

### Details:

PKM2 antibody was used for WB assay on lysates of human colon cancer cells (DLD-1 cells and WiDr cells ) transfected with miR-124 or siR-PTB1 (Fig. 3B and 3C). WB was also performed on lysates after the transfection of colon cancer cells with siR-c-Myc or siR-DDX6 (Fig. 4A and 4B), and on DLD-1 cells /WiDr cells that were subjected to PKM1 and/or PKM2 knockdown using siRNAPKM1 and siRNAPKM2 (Fig. 5B and 5E).

More publications at <http://www.novusbio.com/NBP2-14833>



## Procedures

### Western Blot protocol for PKM1 Antibody (NBP2-14833)

#### Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 25 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 PKM1 Antibody (NBP2-14833)

#### 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 4C.
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.





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Orders: nb-customerservice@bio-techne.com  
General: novus@novusbio.com

### **Products Related to NBP2-14833**

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NB820-59253	Human 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

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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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