

# Product Datasheet

## Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin] NB720-B

Unit Size: 0.5 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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**NB720-B****Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]**

<b>Product Information</b>	
<b>Unit Size</b>	0.5 mg
<b>Concentration</b>	Please see the vial label for concentration. If unlisted please contact technical services.
<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>	0.01% Sodium Azide
<b>Isotype</b>	IgG
<b>Conjugate</b>	Biotin
<b>Purity</b>	Multi-step
<b>Buffer</b>	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2, 10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free

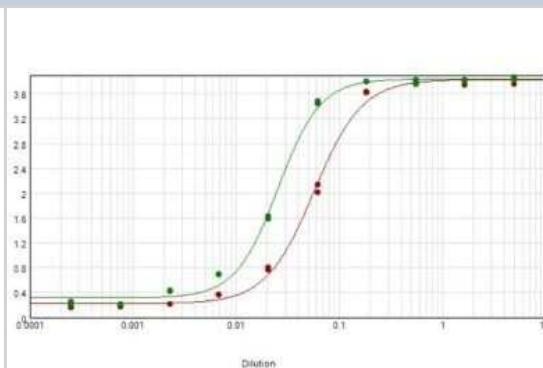
<b>Product Description</b>	
<b>Description</b>	<p>For extended storage aliquot contents and freeze at -20C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room</p> <p>This product was prepared from monospecific antiserum by immunoaffinity chromatography using Mouse IgG coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-biotin, anti-Rabbit Serum, Mouse IgG and Mouse Serum.</p>
<b>Host</b>	Rabbit
<b>Species</b>	Mouse
<b>Immunogen</b>	Mouse IgG whole molecule

<b>Product Application Details</b>	
<b>Applications</b>	Western Blot, ELISA, Immunohistochemistry
<b>Recommended Dilutions</b>	Western Blot 1:20000 - 1:10000, ELISA 1:300000, Immunohistochemistry 1:1000 - 1:5000
<b>Application Notes</b>	This product is available in a variety of formats. Anti-Mouse IgG Biotin Antibody has been tested by ELISA and is suitable for western blot, ELISA and immunohistochemistry as well as other antibody based assays requiring lot-to-lot consistency.



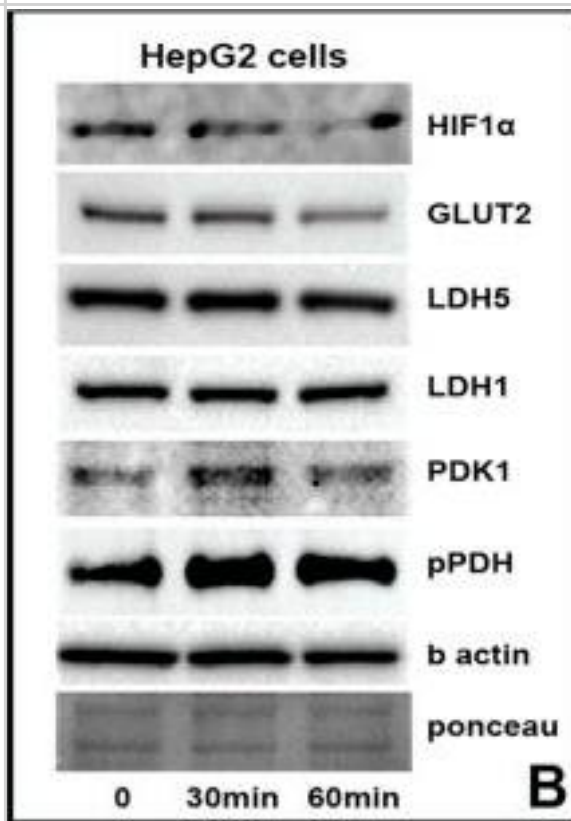
## Images

ELISA: Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin] [NB720-B] - ELISA results of purified Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin] tested against purified Mouse IgG. Each well was coated in duplicate with 1.0 ug of Mouse IgG (green line). The starting dilution of antibody was 5ug/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using blocking buffer, Streptavidin HRP conjugate 1:10000, and TMB substrate.

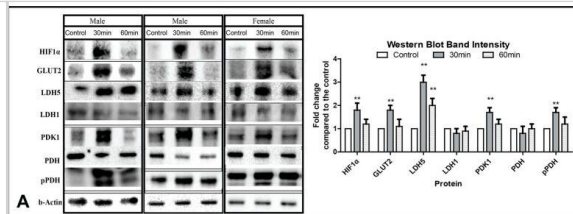


Western Blot: Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin] [NB720-B] - (A) Confocal microscopy of NCTC liver cells before & after exposure to amifostine (100  $\mu\text{g}/\text{ml}$ ), for double PDH(red)/phosphoPDH (green), phosphoPDH(red), LDH5 (red) & HIF1 $\alpha$  (red) expression. Western blot bands following exposure to 100 & 500  $\mu\text{g}/\text{ml}$  of amifostine at 30 min is also shown. (B) Western blot expression of phosphoPDH, PDK1, LDH5 & HIF1 $\alpha$  in hepatoma HepG2 cells, at 0, 30 & 60 min following exposure to 100  $\mu\text{g}/\text{ml}$  of amifostine. (C) Confocal microscopy of NCTC liver cells before & after exposure to amifostine (100  $\mu\text{g}/\text{ml}$ ), for HIF1 $\alpha$  (green), LDH5 (red), PDK1 (green) & phosphoPDH(red) expression with & without silencing of the HIF1 $\alpha$  gene. (D) Acetyl-CoA levels (pmol) in NCTC cells at 0, 30 & 60 min following exposure to 100  $\mu\text{g}/\text{ml}$  of amifostine. (E) ATP levels (pmol) in NCTC cells at 0, 30 & 60 min following exposure to 100  $\mu\text{g}/\text{ml}$  of amifostine. (F) Time course recording of NCTC & HepG2 cell mitochondrial membrane potentials as assessed with the JC1 method & confocal imaging (0–20 minutes), showed a rapid transient reduction of green (monomer) & red (aggregate) forms of the dye that was subsequently restored to normal levels. (G) Mitochondrial ROS (mtROS) production by NCTC & HepG2 cells, after exposure to 18Gy of ionizing radiation with & without pre-incubation with amifostine, showing a strong effect of amifostine in normal NCTC cells. mtROS were low in hepatoma HepG2 cells compared to NCTC hepatocytes & were increased only in dividing neoplastic cells. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/27507219>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

(A) Confocal microscopy of NCTC liver cells before & after exposure to amifostine (100  $\mu\text{g}/\text{ml}$ ), for double PDH(red)/phosphoPDH (green), phosphoPDH(red), LDH5 (red) & HIF1 $\alpha$  (red) expression. Western blot bands following exposure to 100 & 500  $\mu\text{g}/\text{ml}$  of amifostine at 30 min is also shown. (B) Western blot expression of phosphoPDH, PDK1, LDH5 & HIF1 $\alpha$  in hepatoma HepG2 cells, at 0, 30 & 60 min following exposure to 100  $\mu\text{g}/\text{ml}$  of amifostine. (C) Confocal microscopy of NCTC liver cells before & after exposure to amifostine (100  $\mu\text{g}/\text{ml}$ ), for HIF1 $\alpha$  (green), LDH5 (red), PDK1 (green) & phosphoPDH(red) expression with & without silencing of the HIF1 $\alpha$  gene. (D) Acetyl-CoA levels (pmol) in NCTC cells at 0, 30 & 60 min following exposure to 100  $\mu\text{g}/\text{ml}$  of amifostine. (E) ATP levels (pmol) in NCTC cells at 0, 30 & 60 min following exposure to 100  $\mu\text{g}/\text{ml}$  of amifostine. (F) Time course recording of NCTC & HepG2 cell mitochondrial membrane potentials as assessed with the JC1 method & confocal imaging (0–20 minutes), showed a rapid transient reduction of green (monomer) & red (aggregate) forms of the dye that was subsequently restored to normal levels. (G) Mitochondrial ROS (mtROS) production by NCTC & HepG2 cells, after exposure to 18Gy of ionizing radiation with & without pre-incubation with amifostine, showing a strong effect of amifostine in normal NCTC cells. mtROS were low in hepatoma HepG2 cells compared to NCTC hepatocytes & were increased only in dividing neoplastic cells. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/27507219>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin] [NB720-B] - (A) Western blot images & band densitometry analysis of levels of proteins involved in anaerobic metabolism, as assessed in mouse liver before & after administration of amifostine. Bars show standard deviation & asterisks refer to p-values (\* $p < 0.05$ , \*\* $p < 0.001$ ). (B) Confocal immunofluorescent microscopy after staining with anti-PDK1/red antibody showing increased cytoplasmic expression in mouse hepatocytes from 0 (i) to 30 min (ii) & regression thereafter (iii). Double immunostaining with PDH/red & phosphorylated pPDH/green (iv), showed an intensification of the expression of the inactive pPDH form of the enzyme 30 min (v) following amifostine injection & trend for restoration of normal PDH levels at 60 min (vi). Confocal immunofluorescent microscopy after staining for LDH1/red showed stable levels of expression in mouse hepatocytes (i,ii,iii). In contrast, LDH5/red expression was sharply induced 30 min following amifostine injection & decreased thereafter (iv, v, vi). Similar patterns were noted for GLUT2 expression (i,ii,iii) & for HIF1 $\alpha$  expression (iv,v,iv). (C) Analysis of the fluorescence intensity of confocal microscopy images (from five representative tissue areas for each staining). Bars show standard deviation & asterisks refer to p-values (\* $p < 0.05$ , \*\* $p < 0.001$ ). (D) mRNA expression levels of LDHA, PDK1 & GLUT2 (three mice for each time point) following exposure to amifostine, as measured with quantitative RT-PCR. Bars show standard deviation & asterisks refer to p-values (\*\* $p < 0.001$ ). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/27507219>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Ducas AA, Kuhn DCS, Bath LC Et al. Increased matrix metalloproteinase 9 activity correlates with flow-mediated intraluminal thrombus deposition and wall degeneration in human abdominal aortic aneurysm *JVS Vasc Sci* 2021-10-07 [PMID: 34617048]

Details:

Citation using the Biotin version of this antibody.

Koukourakis MI, Giatromanolaki A, Zois CE et al. Normal tissue radioprotection by amifostine via Warburg-type effects *Sci Rep* 2016-08-11 [PMID: 27507219] (WB)



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### **Products Related to NB720-B**

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NBP2-29370	Streptavidin Native Protein
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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Secondary Antibodies are guaranteed for 1 year from date of receipt.

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