

# Product Datasheet

## CD36 Antibody - BSA Free NB400-144

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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**NB400-144**

CD36 Antibody - BSA Free

**Product Information**

<b>Unit Size</b>	0.1 ml
<b>Concentration</b>	1 mg/ml
<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>	0.02% Sodium Azide
<b>Isotype</b>	IgG
<b>Purity</b>	Immunogen affinity purified
<b>Buffer</b>	PBS
<b>Target Molecular Weight</b>	110 kDa

**Product Description**

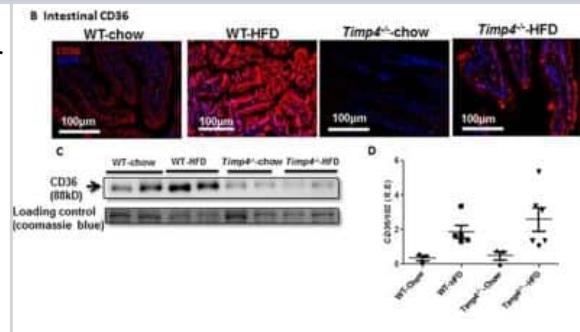
<b>Description</b>	Novus Biologicals Rabbit CD36 Antibody - BSA Free (NB400-144) is a polyclonal antibody validated for use in IHC, WB, ELISA and ICC/IF. Anti-CD36 Antibody: Cited in 130 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
<b>Host</b>	Rabbit
<b>Gene ID</b>	948
<b>Gene Symbol</b>	CD36
<b>Species</b>	Human, Mouse, Rat, Porcine, Avian, Bovine, Primate, Rabbit
<b>Reactivity Notes</b>	Use in Mouse reported in scientific literature (PMID:34974159) Porcine reactivity reported in scientific literature (PMID: 23727393). Rat reactivity reported in scientific literature (PMID: 25635851). Rabbit reactivity reported in scientific literature (PMID: 30105261). Avian reactivity reported from a verified customer review.
<b>Marker</b>	Endothelial Cell Marker
<b>Immunogen</b>	This CD36 Antibody was developed against a synthetic peptide mapping to a region of human CD36 between residues 100-200 [Uniprot# P16671]

**Product Application Details**

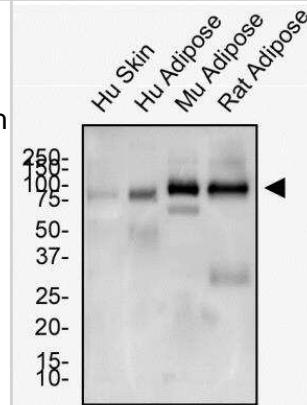
<b>Applications</b>	Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen
<b>Recommended Dilutions</b>	Western Blot 1:500-1:2000, Immunohistochemistry 1:200-1:400, Immunocytochemistry/ Immunofluorescence 1:50-1:200, Immunohistochemistry-Paraffin 1:200-1:400, Immunohistochemistry-Frozen reported in scientific literature (PMID 24531551)
<b>Application Notes</b>	In Western Blot, a band is seen ~75-80 kDa. The theoretical molecular weight of CD36 is ~53 kDa. The difference in theoretical MW and actual MW as seen in Western blot is most likely due to the heavy glycosylation and palmitoylation of this protein. 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

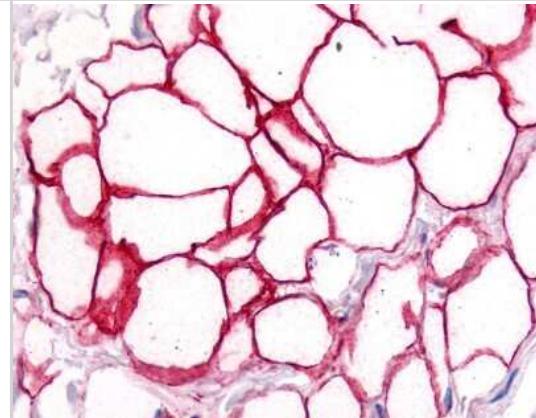
Immunohistochemistry: CD36 Antibody [NB400-144] - Timp4-deficiency results in defective lipid digestion and absorption. (B) Immunostaining for CD36 in small intestine (proximal region) of chow-fed and HFD-fed WT and Timp4-/- mice. Western blot (C) and mRNA (D) for CD36 in enterocyte fraction of chow-fed and HFD-fed WT and Timp4-/- mice (collected from the proximal small intestine). Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41598-017-05951-4>), licensed under a CC-BY license.



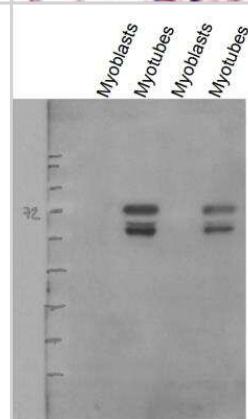
Western Blot: CD36 Antibody [NB400-144] - Total protein from Human Skin and Adipose tissue, Mouse Adipose and Rat Adipose tissue was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-CD36 in 5% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.



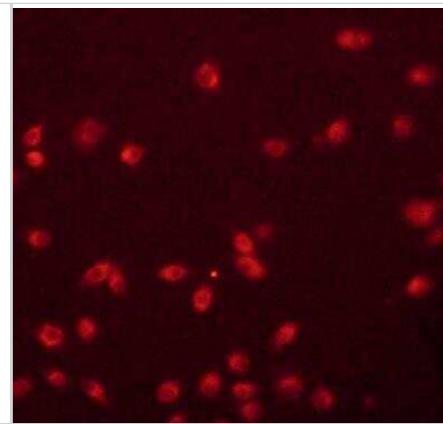
Immunohistochemistry-Paraffin: CD36 Antibody [NB400-144] - Staining of CD36 in human adipocytes.



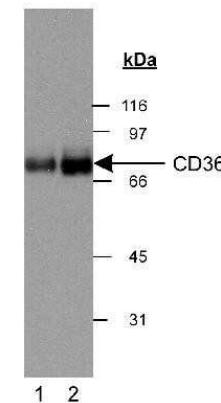
Western Blot: CD36 Antibody [NB400-144] - Mouse primary muscle cell lysate. Western blot image submitted by a verified customer review.



Immunocytochemistry/Immunofluorescence: CD36 Antibody [NB400-144] - Bone marrow derived mouse macrophages stained with CD36 antibody. ICC/IF image submitted by a verified customer review.



Western Blot: CD36 Antibody [NB400-144] - Detection of CD36 in human adipocyte extract (30 ug). Lane 1: 0.5 ug/ml NB 400-144; lane 2: 2 ug/ml NB 400-144. ECL: 3 second exposure.



ATM activation by chloroquine alleviates senescence. (a) Immunoblots showing protein levels of ATM, NBS1, and RAP80 in human skin fibroblasts (HSFs). A gradually increased level of p16 indicates cellular senescence, while elevated  $\gamma$ H2AX level indicates accumulated DNA damage. (b) Immunoblots showing protein levels of ATM, NBS1, and RAP80 in mouse embryonic fibroblasts (MEFs). (c) Immunoblots showing protein levels of ATM, NBS1, and RAP80 in brain tissues isolated from 3-, 10-, and 18-month-old male mice. (d) SA- $\beta$ -Gal staining in HSFs treated with sh-ATM or scramble shRNA. Scale bar, 100  $\mu$ m. (e) Quantification of SA- $\beta$ -Gal-positive staining of (d) from five views randomly captured for each group. Data represent means  $\pm$  SEM.

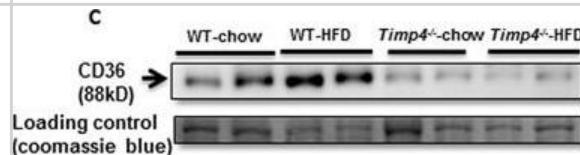
\*\*\*p<0.001. (f) Immunoblots showing increased  $\gamma$ H2AX and unaffected LC3I/II in HSFs treated with sh-ATM or scramble shRNA. (g) Immunoblots showing protein levels of pS1981 ATM,  $\gamma$ H2AX, and cleaved caspase-3 in HSFs treated with 10  $\mu$ M of CQ for indicated time.

(h) SA- $\beta$ -Gal staining in HSFs expressing either scramble or ATM shRNA treated with 1  $\mu$ M CQ or DMSO (12 hr). Scale bar, 100  $\mu$ m. (i) Quantification of SA- $\beta$ -Gal-positive staining of (h) from five views randomly captured for each group. Data represent means  $\pm$  SEM.

\*\*\*p<0.001; 'N.S.' indicates no significant difference. (j) HSFs at passage 20 were continuously cultured with 1  $\mu$ M CQ or DMSO, and cell number was calculated at each passage. Data represent means  $\pm$  SEM.

\*\*\*p<0.01. (k) Immunoblots showing protein levels of  $\gamma$ H2AX, p62, and LC3 in MEFs treated with 1  $\mu$ M CQ or DMSO. Note that CQ had little effect on the expression levels of p62 and LC3. (l) MEFs at passage one were continuously cultured in 20% O<sub>2</sub> with 1  $\mu$ M CQ or DMSO, and cell number was determined at each passage. Data represent means  $\pm$  SEM.

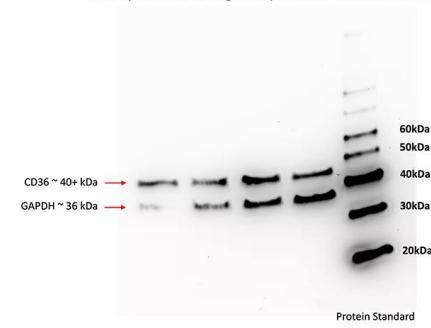
\*\*\*p<0.01. 10.7554/eLife.34836.006 Figure 1—source data 1. Statistical analysis for SA- $\beta$ -Gal positive staining. 10.7554/eLife.34836.007 Figure 1—source data 2. Statistical analysis for EdU positive staining. Statistical analysis for SA- $\beta$ -Gal positive staining. Statistical analysis for EdU positive staining. Decline of ATM-centered DNA repair machinery during senescence. (a) Real-time PCR analysis showing progressively elevated



mRNA level of p21 in continuously cultured human endothelial cells (HUVEC). \*\*p<0.01. (b) SA- $\beta$ -Gal staining of HUVEC cells at indicated passages. Scale bar, 100  $\mu$ m. (c) HUVEC cells at P21, P18, P12, and P7 were subjected to transcriptome analysis. A minimum average rpkm value of 1.0 and maximum 10% fluctuation in young cells (P7 Vs P12) was set as the threshold. Genes were downregulated by more than 20% in pre-senescence, and senescent cells compared with young cells (P21/P18 Vs P12/P7) were selected. (d) Pathway analysis of genes identified in (c) by STRING v10. (e) Downregulation of ATM-related DNA repair genes during senescence. ATM regulates replicative senescence. (a) Representative images showing cells treated with Scramble (sh-NC) or sh-ATM. (b) Percent EdU-positive cells in sh-NC or sh-ATM treated HSFs. Views were randomly captured and at least 100 cells were included in each group. Data represent means  $\pm$  SEM. \*\*\*p<0.001. (c) Immunoblots showing protein levels of pS1981 ATM and  $\gamma$ H2AX in HSFs treated with 10  $\mu$ M chloroquine (CQ) or 0.4  $\mu$ M CPT (4 hr). Note that CQ activated ATM (pS1981) without increasing  $\gamma$ H2AX, while CPT activated ATM accompanied by increased  $\gamma$ H2AX. (d) SA- $\beta$ -Gal staining in primary MEFs treated with 1  $\mu$ M CQ or DMSO. Scale bar, 100  $\mu$ m. (e) Quantification of SA- $\beta$ -Gal-positive staining of (d) from five views randomly captured for each group. Data represent means  $\pm$  SEM. \*\*\*p<0.001. (f) Percent EdU-positive cells in HSFs treated with DMSO, 1  $\mu$ M or 10  $\mu$ M CQ. Views were randomly captured and at least 100 cells were included in each group. Data represent means  $\pm$  SEM. \*\*\*p<0.001. (g) Representative images showing proliferative HSFs treated with different doses of CQ for the indicated time points. (h) Immunoblots showing LC3B levels in HSFs treated with indicated dose of CQ for indicated period of time. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/29717979/>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Western Blot: Rabbit Polyclonal CD36 Antibody [NB400-144] - The western blot was conducted on avian (passerine) skeletal muscle tissue homogenate. 6ug of total protein was loaded and GAPDH was probed to ensure total protein loads were similar across samples. Rabbit polyclonal CD36 antibody (NB400-144) was used at 1:5000 dilution (diluted in 5% milk). CD36 and GAPDH were probed sequentially - not multiplexed. CD36 produced a band above 40kDa and GAPDH at 36kDa. Image from a verified customer review.

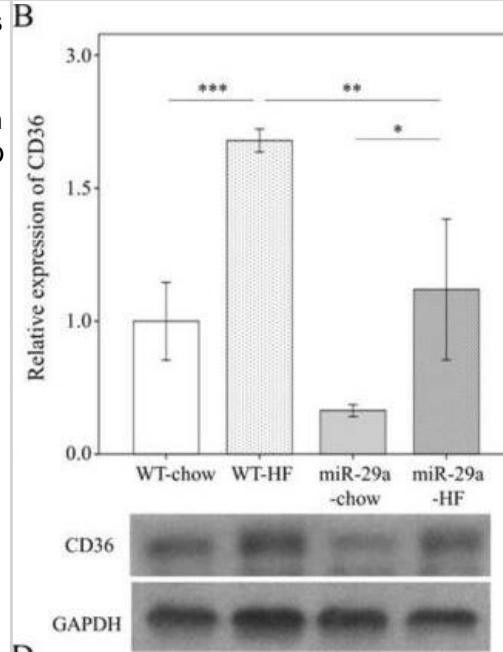
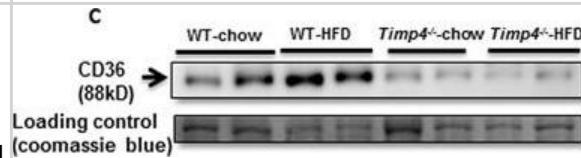
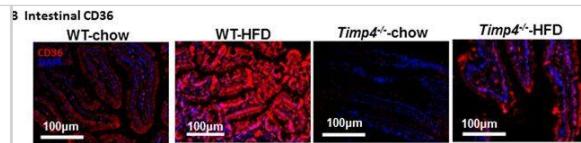
Avian pectoralis homogenate probed for CD36 and GAPDH



Immunocytochemistry/ Immunofluorescence: CD36 Antibody - BSA Free [NB400-144] - Timp4-deficiency results in defective lipid digestion & absorption. Fecal triglyceride (TG) content (Ai) in chow- or HFD-fed WT & Timp4<sup>-/-</sup> mice. Fecal lipid analysis by thin layer chromatography in chow-fed (Aii) & HFD-fed (iii) WT & Timp4<sup>-/-</sup> mice. (B) Immunostaining for CD36 in small intestine (proximal region) of chow-fed & HFD-fed WT & Timp4<sup>-/-</sup> mice. Western blot (C) & mRNA (D) levels for CD36 in enterocyte fraction of chow-fed & HFD-fed WT & Timp4<sup>-/-</sup> mice (collected from the proximal small intestine). (E) Triglyceride levels in enterocytes collected from the proximal small intestine. Averaged data are presented as mean  $\pm$  S.E.M & analyzed by ANOVA. \* indicates significance ( $p < 0.05$ ). Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41598-017-05951-4>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

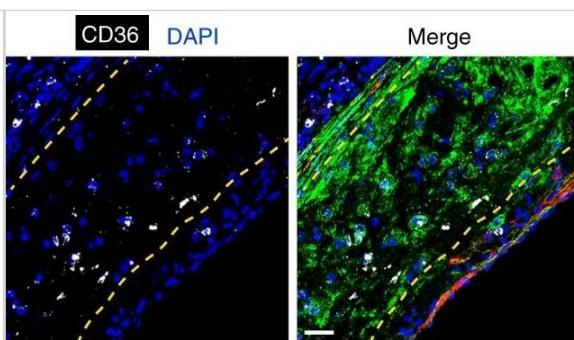
Western Blot: CD36 Antibody - BSA Free [NB400-144] - Timp4-deficiency results in defective lipid digestion & absorption. Fecal triglyceride (TG) content (Ai) in chow- or HFD-fed WT & Timp4<sup>-/-</sup> mice. Fecal lipid analysis by thin layer chromatography in chow-fed (Aii) & HFD-fed (iii) WT & Timp4<sup>-/-</sup> mice. (B) Immunostaining for CD36 in small intestine (proximal region) of chow-fed & HFD-fed WT & Timp4<sup>-/-</sup> mice. Western blot (C) & mRNA (D) levels for CD36 in enterocyte fraction of chow-fed & HFD-fed WT & Timp4<sup>-/-</sup> mice (collected from the proximal small intestine). (E) Triglyceride levels in enterocytes collected from the proximal small intestine. Averaged data are presented as mean  $\pm$  S.E.M & analyzed by ANOVA. \* indicates significance ( $p < 0.05$ ). Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41598-017-05951-4>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Western Blot: CD36 Antibody - BSA Free [NB400-144] - MiR-29a inhibits the expression of fatty acid translocase CD36 by targeting 3' untranslated region (UTR). (A) qPCR analysis of Cd36 in live tissue. (B) Representative immunoblotting bands & densitometric results of CD36 in liver tissue. (C) qPCR analysis of cd36 expression of HepG2 cells in vitro after 48h transfection of scramble sequence or miR-29a-mimic. Data obtained from six independent experiments. (D) Upper panel, sequence information, & mutual pairing status of CD36-3'UTR, mmu-miR29a, & CD36-3'UTR Mut. Note that red characters represent mismatching sites. HepG2 was first transfected with CD36-3'UTR or CD36-3'UTR mutant luciferase reporter construct then treated with control medium (ctrl), miRNA-scramble, or miR-29a mimic, & finally lysed to detect the luciferase signal. Data are expressed as mean  $\pm$  SE. \*  $p < 0.05$ , \*\*  $p < 0.01$ , & \*\*\*  $p < 0.001$  between the indicated groups. WT, wild type mice. HFD, high-fat diet. miR-29a, mice harboring overexpression of miR-29a. mmu-miR29a, mouse-origin miR-29a. ctrl, control. Mut, mutant. UTR, untranslated region. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31652636>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

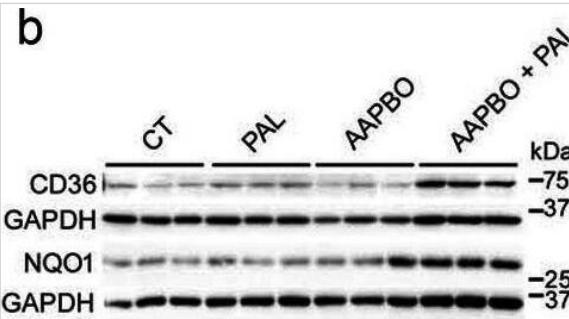


Immunohistochemistry: CD36 Antibody - BSA Free [NB400-144] - Integrin  $\beta$ 3 modulates SMC transdifferentiation. a-c Mice were fed a HFD for 6 or 16 weeks as indicated, & then transverse aortic root sections were stained. In a, b sections from ApoE(-/-), SMMHC-CreERT2, ROSA26R(mTmG+) mice were stained for SMA, GFP (fate marker), nuclei (DAPI), & either integrin  $\beta$ 3 (a) or CD36 (b). Dashed yellow lines separate cap from core (a, b) & core from media (b). n = 5. In c sections from ApoE(-/-) mice that were also wild type or null for Itgb3 were stained for SMMHC, CD68, & nuclei (DAPI). n = 3. Boxed regions (a, c) are shown as close-ups on right; in c CD68+SMMHC+ cells in the media (arrowheads) & plaque (arrows) of the Itgb3 null atherosclerotic aorta are indicated. Med, tunica media; Lu, lumen; Pl, plaque. Scale bars, 25  $\mu$ m. d-h Aortic SMCs were isolated from ApoE(-/-) mice & then subjected to siRNA-mediated knockdown with si-Itgb3 vs. scrambled (Scr; d-f) or with si-Itgb3, si-Tlr4 vs. si-Itgb3 (g, h). Levels of indicated transcripts from qRT-PCR are relative to Gapdh & normalized to either Scr in d, f or to si-Itgb3 in g, h. For d, g, n = 4-5 in duplicate. In e silenced SMCs were cultured with Dil-conjugated ox-LDL for 10 h & stained with DAPI; n = 5. In f, h silenced SMCs were exposed to soluble cholesterol:methyl- $\beta$ -cyclodextrin complexes for 3 days, & then mRNA levels were assessed; n = 4-7 in duplicate. \*, \*\*, \*\*\*, ^, \*\*\*\* vs. control (Scr in d, f & si-Itgb3 in g, h), p < 0.05, p < 0.01, p < 0.005, p < 0.001, & p < 0.0005, respectively. NS, not significant. Student's t-test was used, & error bars represent standard deviations. i Schematic of the effect of  $\beta$ 3 reduction via TLR4 & CD36 in SMCs on transdifferentiation Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/s41467-018-04447-7>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Western Blot: CD36 Antibody - BSA Free [NB400-144] - The increase in CD36 levels caused by lipids in Sirt3-deficient hepatocytes is mediated by Nrf2. VLDLR mRNA abundance (a) & protein levels of VLDLR & NQO1, an Nrf-2-target gene, (b) were assessed in Huh-7 cells incubated with fatty acid free-BSA or BSA-palmitate (0.3 mM) & exposed to either vehicle or the Sirt3 inhibitor AAPBO (100  $\mu$ M) for 16 h. a, p < 0.05 vs. CT. b, p < 0.05 vs. CT cells incubated with palmitate. c, p < 0.05 vs. CT cells treated with AAPBO. c, fatty acid uptake in Huh-7 cells incubated with fatty acid free-BSA or BSA-palmitate (0.3 mM) & exposed to either vehicle or the Sirt3 inhibitor AAPBO (100  $\mu$ M) for 16 h was measured by the uptake of BODIPY-C16. a, p < 0.05 vs. CT. b, p < 0.05 vs. CT cells incubated with palmitate. c, p < 0.05 vs. CT cells treated with AAPBO. mRNA abundance (d) & protein levels of VLDLR (e) in Huh-7 cells transfected with control (CT) or SIRT3 siRNA & incubated in the presence or absence 0.3 mM palmitate (Pal) for 24 h. Protein levels of CD36 (f), NQO1 (g) & PPAR $\gamma$  (h) in Huh-7 cells transfected with control (CT) or SIRT3 siRNA & incubated in the presence or absence 0.3 mM palmitate (Pal) or the Nrf2 inhibitor ML385 (10  $\mu$ M) for 24 h. a, p < 0.05 vs. CT siRNA cells. b, p < 0.05 vs. CT siRNA cells incubated with palmitate. c, p < 0.05 vs. SIRT3 siRNA cells. d, p < 0.05 vs. CT siRNA cells incubated with palmitate & ML385. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32912335>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: CD36 Antibody - BSA Free [NB400-144] - The increase in CD36 levels caused by lipids in Sirt3-deficient hepatocytes is mediated by Nrf2. VLDLR mRNA abundance (a) & protein levels of VLDLR & NQO1, an Nrf-2-target gene, (b) were assessed in Huh-7 cells incubated with fatty acid free-BSA or BSA-palmitate (0.3 mM) & exposed to either vehicle or the Sirt3 inhibitor AAPBO (100  $\mu$ M) for 16 h. a, p < 0.05 vs. CT. b, p < 0.05 vs. CT cells incubated with palmitate. c, p < 0.05 vs. CT cells treated with AAPBO. c, fatty acid uptake in Huh-7 cells incubated with fatty acid free-BSA or BSA-palmitate (0.3 mM) & exposed to either vehicle or the Sirt3 inhibitor AAPBO (100  $\mu$ M) for 16 h was measured by the uptake of BODIPY-C16. a, p < 0.05 vs. CT. b, p < 0.05 vs. CT cells incubated with palmitate. c, p < 0.05 vs. CT cells treated with AAPBO. mRNA abundance (d) & protein levels of VLDLR (e) in Huh-7 cells transfected with control (CT) or SIRT3 siRNA & incubated in the presence or absence 0.3 mM palmitate (Pal) for 24 h. Protein levels of CD36 (f), NQO1 (g) & PPAR $\gamma$  (h) in Huh-7 cells transfected with control (CT) or SIRT3 siRNA & incubated in the presence or absence 0.3 mM palmitate (Pal) or the Nrf2 inhibitor ML385 (10  $\mu$ M) for 24 h. a, p < 0.05 vs. CT siRNA cells. b, p < 0.05 vs. CT siRNA cells incubated with palmitate. c, p < 0.05 vs. SIRT3 siRNA cells. d, p < 0.05 vs. CT siRNA cells incubated with palmitate & ML385. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32912335/>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Rey E, Meléndez-Rodríguez F, Marañón P et al. Hypoxia-inducible factor 2? drives hepatosteatosis through the fatty acid translocase CD36 *Liver International* 2020-10-01 [PMID: 32432822] (Western Blot, Mouse)

Ma Y, Huang L, Zhang Z et al. CD36 promotes tubular ferroptosis by regulating the ubiquitination of FSP1 in acute kidney injury *Genes & Diseases* 2024-01-01 [PMID: 37588197] (Western Blot, Mouse)

Yang YL, Wang PW, Wang FS et al. miR-29a Modulates GSK3?/SIRT1-Linked Mitochondrial Proteostatic Stress to Ameliorate Mouse Non-Alcoholic Steatohepatitis *International Journal of Molecular Sciences* 2020-09-19 [PMID: 32961796] (Western Blot, Mouse)

Aoki T, Kinoshita J, Munesue S et al. Hypoxia-Induced CD36 Expression in Gastric Cancer Cells Promotes Peritoneal Metastasis via Fatty Acid Uptake *Annals of Surgical Oncology* 2023-05-01 [PMID: 36042102] (Western Blot, Mouse)

You M, Wu F, Gao M et al. Selenoprotein K contributes to CD36 subcellular trafficking in hepatocytes by accelerating nascent COPII vesicle formation and aggravates hepatic steatosis *Redox Biology* 2022-11-01 [PMID: 36252341] (Western Blot, Mouse)

Sfyri PP, Yuldasheva NY, Tzimou A et al. Attenuation of oxidative stress-induced lesions in skeletal muscle in a mouse model of obesity-independent hyperlipidaemia and atherosclerosis through the inhibition of Nox2 activity *Free Radical Biology and Medicine* 2018-12-01 [PMID: 30342191] (Western Blot, Mouse)

Luo X, Zheng E, Wei L et al. The fatty acid receptor CD36 promotes HCC progression through activating Src/PI3K/AKT axis-dependent aerobic glycolysis *Cell Death & Disease* 2021-03-26 [PMID: 33771982] (Western Blot, Mouse)

Choi Y, Song MJ, Jung WJ et al. Liver-Specific Deletion of Mouse CTCF Leads to Hepatic Steatosis via Augmented PPAR? Signaling *Cellular and Molecular Gastroenterology and Hepatology* 2021-08-04 [PMID: 34358714] (Western Blot, Mouse)

Boso D, Tognon M, Curtarello M et al. Anti-VEGF therapy selects for clones resistant to glucose starvation in ovarian cancer xenografts *Journal of Experimental & Clinical Cancer Research* 2023-08-07 [PMID: 37550722] (Western Blot, Mouse)

Overby H, Yang Y, Xu X et al. Soluble Epoxide Hydrolase Inhibition by t-TUCB Promotes Brown Adipogenesis and Reduces Serum Triglycerides in Diet-Induced Obesity *International Journal of Molecular Sciences* 2020-09-24 [PMID: 32987880] (Western Blot, Mouse)

Yang Y, Xu X, Wu H et al. Differential Effects of 17,18-EEQ and 19,20-EDP Combined with Soluble Epoxide Hydrolase Inhibitor t-TUCB on Diet-Induced Obesity in Mice *International Journal of Molecular Sciences* 2021-07-31 [PMID: 34361032] (Western Blot, Mouse)

Luo L, Liu Y, Nizigiyimana P et al. DNA 6mA Demethylase ALKBH1 Orchestrates Fatty Acid Metabolism and Suppresses Diet-Induced Hepatic Steatosis *Cellular and Molecular Gastroenterology and Hepatology* 2022-09-02 [PMID: 36058506] (Western Blot, Mouse)

More publications at <http://www.novusbio.com/NB400-144>

## Procedures

### Western Blot Protocol for CD36 Antibody (NB400-144)

#### Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST 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 manufacturer's instructions.

### Immunocytochemistry/Immunofluorescence Protocol for CD36 Antibody (NB400-144)

#### 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. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization 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.

**Immunohistochemistry-Paraffin Protocol for CD36 Antibody (NB400-144)****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 (keep slides in the sodium citrate buffer at all times).

**Staining:**

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) 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 HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.



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## Products Related to NB400-144

NBL1-08939	CD36 Overexpression Lysate
NB400-144PEP	CD36 Antibody Blocking Peptide
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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