Product Datasheet

CD36 Antibody (1283D) - BSA Free
NBP2-54790

Unit Size: 0.1 mg

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

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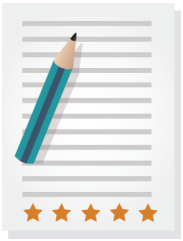
Updated 10/23/2024 v.20.1



NBP2-54790

CD36 Antibody (1283D) - BSA Free

Product Information

Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	1283D
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Protein A or G purified
Buffer	PBS
Target Molecular Weight	110 kDa

Product Description

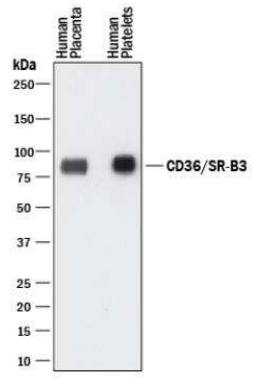
Host	Rabbit
Gene ID	948
Gene Symbol	CD36
Species	Human
Immunogen	This CD36 Antibody (1283D) was developed against a synthetic peptide made to an internal portion of the human CD36 protein (between amino acids 100-150) [Uniprot P16671]

Product Application Details

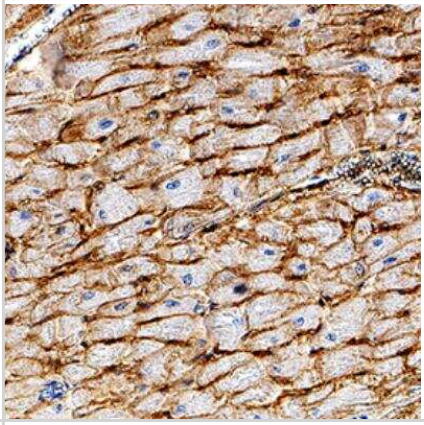
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1.0 ug/ml, Immunohistochemistry 5-10 ug/ml, Immunocytochemistry/ Immunofluorescence 10-20 ug/ml, Immunohistochemistry-Paraffin 5-10 ug/ml

Images

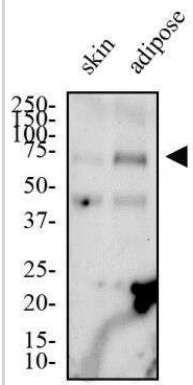
Western Blot: CD36 Antibody (1283D) [NBP2-54790] - Western blot shows lysates of human placenta tissue and human platelets. PVDF membrane was probed with 1 ug/ml of Rabbit Anti-Human CD36 Monoclonal antibody (catalog number NBP2-54790) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (catalog number HAF008). A specific band was detected for CD36/SR-B3 at approximately 85 kDa (as indicated). This experiment was conducted under reducing conditions.



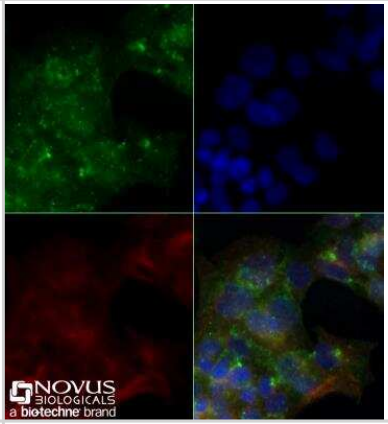
Immunohistochemistry-Paraffin: CD36 Antibody (1283D) [NBP2-54790] - CD36 was detected in immersion fixed paraffin-embedded sections of human heart using Rabbit Anti-Human CD36/SR-B3 Monoclonal Antibody for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte(TM) HRP Polymer Antibody. Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cardiomyocyte membranes.



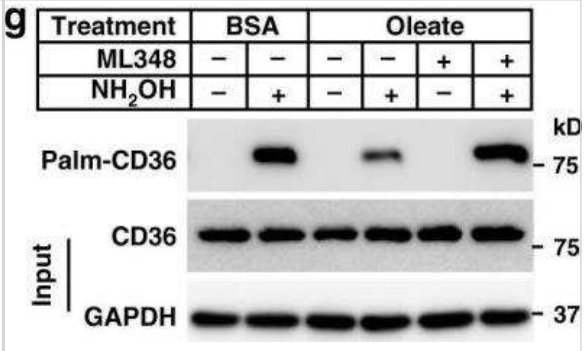
Western Blot: CD36 Antibody (1283D) [NBP2-54790] - Total protein from human skin and adipose tissue was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 1.0 ug/ml anti-CD36 in 1% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.



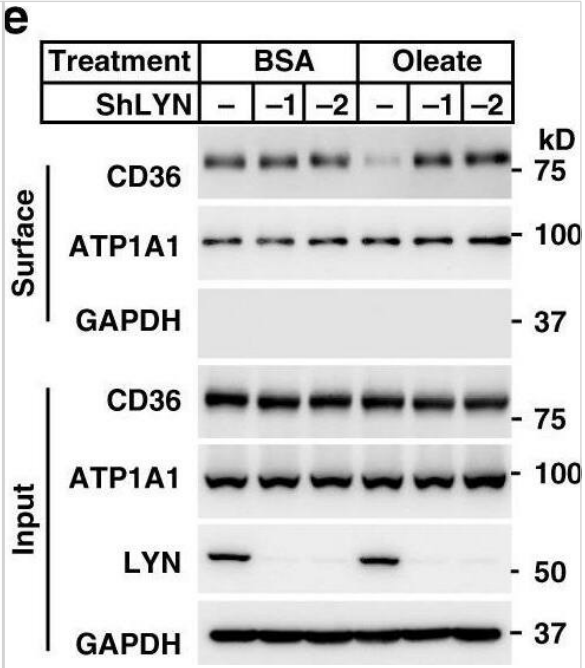
Immunocytochemistry/Immunofluorescence: CD36 Antibody (1283D) [NBP2-54790] - HepG2 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti-CD36 (1283D) at 5.0 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective..



Western Blot: CD36 Antibody (1283D) - BSA Free [NBP2-54790] - 3T3-L1 adipocytes were pretreated and treated with ML348 (10 uM) for 1 h, followed by BSA or oleate treatment for another 1 h. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/32958780/) licensed under a CC-BY license.



Western Blot: CD36 Antibody (1283D) - BSA Free [NBP2-54790] - LYN phosphorylates DHHC5 & is required for the endocytosis of FAs.a 3T3-L1 adipocytes pretreated with serum-free medium for 4 h & PP2 (15 μM) for 1 h, followed by treatment with BSA or oleate (100 μM) for 5 min. Cells harvested & immunoprecipitated with anti-DHHC5 antibody to detect phosphorylation of Tyr91. b 3T3-L1 adipocytes transduced with indicated shRNA set up & treated with oleate for 5 min. Cells harvested to detect Tyr91 phosphorylation as in (a). c On day 0, HEK293T cells set up at 2.5 × 105 cells per 6-cm dish. On day 2, cells transfected with 0.5 μg of WT, Y91E, or Y91F of DHHC5-Flag/pCDH-puro and/or 0.5 μg LYN/pCDNA3.3. On day 3, cells harvested & immunoprecipitated with anti-Flag M2 beads to detect phosphorylation of DHHC5 (pY). Control & LYN knockdown adipocytes pretreated & treated with BSA or oleate (100 μM) for 1 h, followed by immunostaining (d), surface biotinylation (e), & Acyl-RAC (f) assays. Control & CD36 knockdown 3T3-L1 adipocytes pretreated & treated with oleate (100 μM) for indicated time (g) or 5 min (h). Cells harvested & subjected to immunoprecipitation of LYN to detect phosphorylation of LYN (Y396). i, j WT & Cd36−/− SVFs set up & subjected to FA uptake as in Fig. 3h, i, except that cells pretreated with PP2 (15 μM). Each value represents mean ± SEM obtained from 20 cells. Two-sided Student's t test was performed between DMSO & PP2 treated cells. k WT & Cd36−/− adipocytes set up & subjected to 3H-oleate uptake as in Fig. 3j, except that cells pretreated with PP2 (15 μM). Each value represents mean ± SEM obtained from 3samples. Two-sided Student's t test was performed between DMSO & PP2 treated cells. These experiments repeated at least twice. Source data provided as a Source Data file. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32958780), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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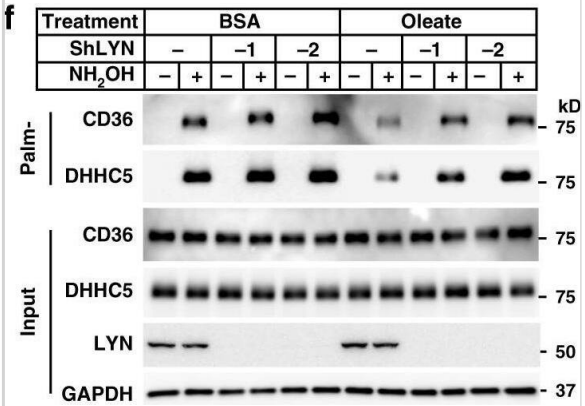
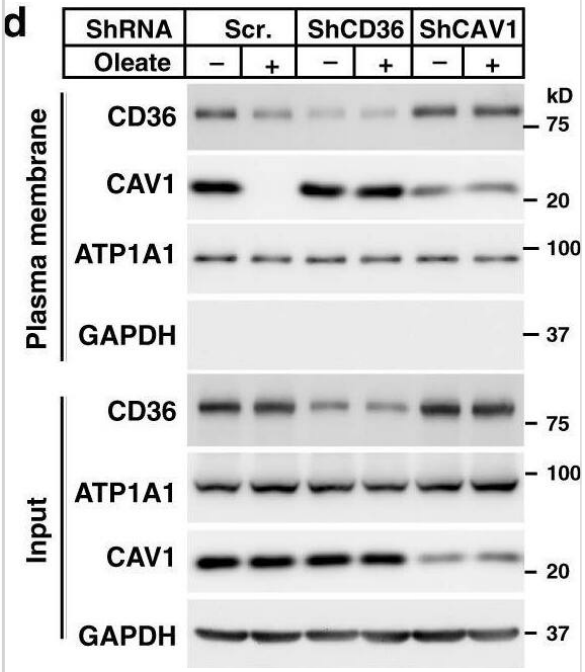


Western Blot: CD36 Antibody (1283D) - BSA Free [NBP2-54790] - FAs trigger internalization of CD36.a, b On day 8 of differentiation, 3T3-L1 adipocytes were pretreated with serum-free medium for 4 h, & then treated with BSA-conjugated oleate (100 μM) for indicated time. a One set of cells was subjected to immunostaining with anti-CD36 & anti-CAV1 antibodies. LipidTOX was used to label lipid droplets. Images were taken under a Zeiss LSM-780 microscope in a 3D Z-stack mode & reconstructed using Imaris 9.2.0. b The other set of cells was subjected to surface biotinylation assay & blotted with indicated antibodies. c, d On day 4 of differentiation, 3T3-L1 cells were infected with lentivirus encoding scrambled shRNA or shRNAs against CD36 or CAV1. On day 5, cells were selected with 5 μg/ml puromycin. On day 8, cells were pretreated as in (a) & treated with oleate (100 μM) for 4 h, followed by immunostaining with anti-CD36 & anti-CAV1 antibodies (c), or surface biotinylation assay (d). e, f 3T3-L1 adipocytes were pretreated as in (a) & treated with BSA-conjugated FAs with different chain lengths or saturation (100 μM) for 4 h. Cells were subjected to immunostaining with anti-CD36 antibody (e), or surface biotinylation assay (f). After oleate treatment for 4 h, 3T3-L1 adipocytes were switched to serum-free medium for indicated time & harvested for immunostaining (g) & surface biotinylation (h). The scale bars were as indicated in each figure. These experiments were repeated at least three times. Source data are provided as a Source Data file. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32958780), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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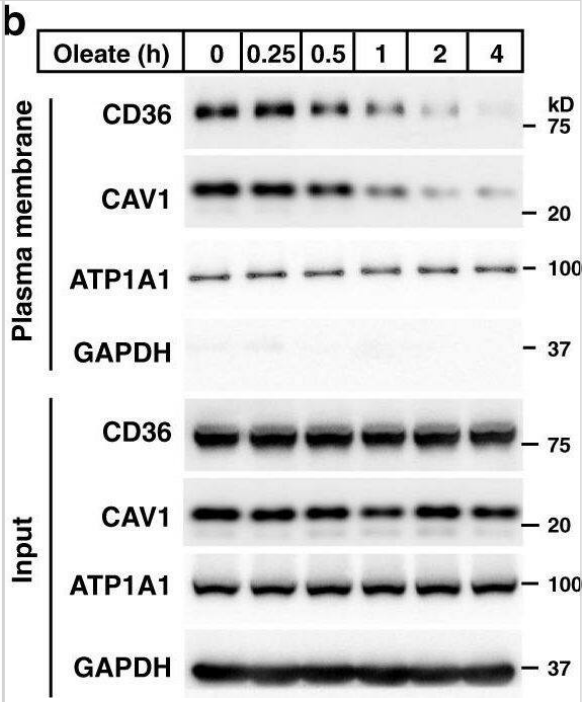

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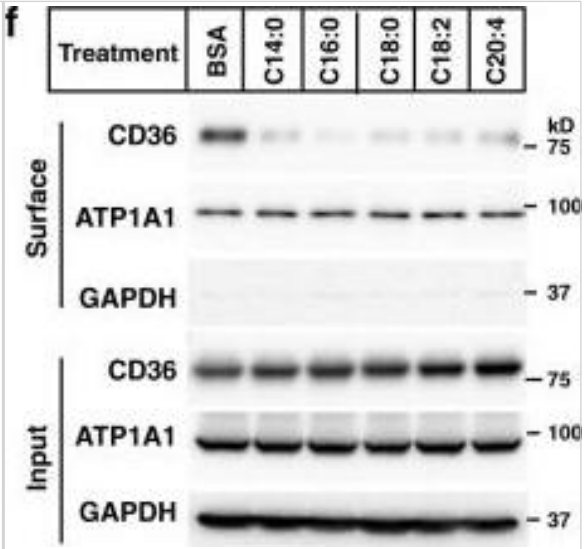

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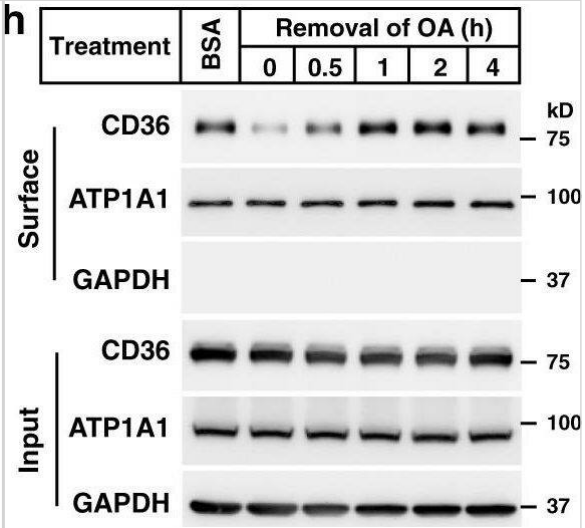
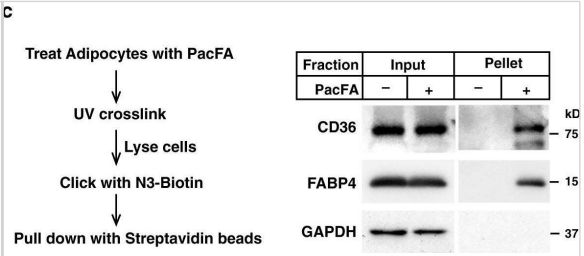


Western Blot: CD36 Antibody (1283D) - BSA Free [NBP2-54790] - CD36 -mediated caveolar endocytosis transports FAs into cells.a–c 3T3-L1 adipocytes were pretreated with serum-free medium for 4 h, & then treated with BSA-conjugated PacFA (50 μM) for 20 min, followed by UV crosslinking on ice for 30 min. a, b Cells were subjected to click chemistry using an N3-Alexa Fluro 488, & immunostained with anti-CD36 antibody. Colocalization of PacFA & CD36 was quantified from 24 cells over three independent experiments & plotted in (b). The value represents mean ± SEM. c Cells were lysed & subjected to click chemistry assay using N3-biotin. PacFA-labeled proteins were captured with streptavidin beads & subjected to western blot using anti-CD36 & anti-FABP4 antibodies. d WT, Cav1−/−, Cd36−/−, & Cav1−/−;Cd36−/− SVFs were isolated & differentiated into adipocytes & treated with 100 μM 3H-oleate (specific activity, 2268 dpm/nmol) for 1 h. Lipid fractions were extracted from the cells & subjected to scintillation counting. The radioactive counting was normalized to protein content. Each value represents mean ± SEM obtained from three samples. Two-sided Student's t test was performed between WT & each of the knockout cells. These experiments were repeated twice. Source data are provided as a Source Data file. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32958780), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Hao JW, Wang J, Guo H et al. CD36 facilitates fatty acid uptake by dynamic palmitoylation-regulated endocytosis Nat Commun 2020-09-21 [PMID: 32958780] (IP)

Procedures

Western Blot Protocol for CD36/SR-B3 Antibody (NBP2-54790)

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 anti-CD36 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%.

Immunocytochemistry Protocol for CD36/SR-B3 Antibody (NBP2-54790)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

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

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Products Related to NBP2-54790

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NB400-145PEP	CD36 Antibody Blocking Peptide

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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