Product Datasheet

Perilipin-2/ADFP Antibody - BSA Free
NB110-40877

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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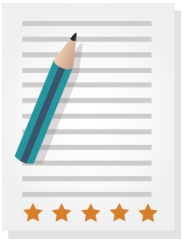
Updated 12/20/2023 v.20.1



NB110-40877

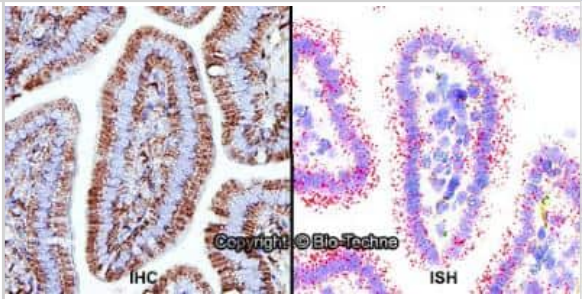
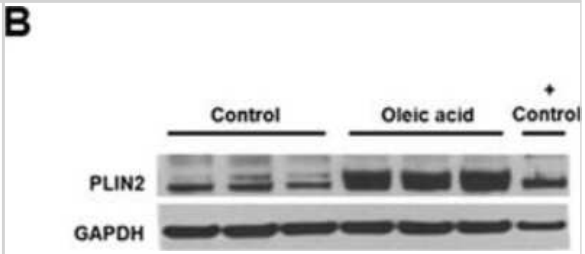
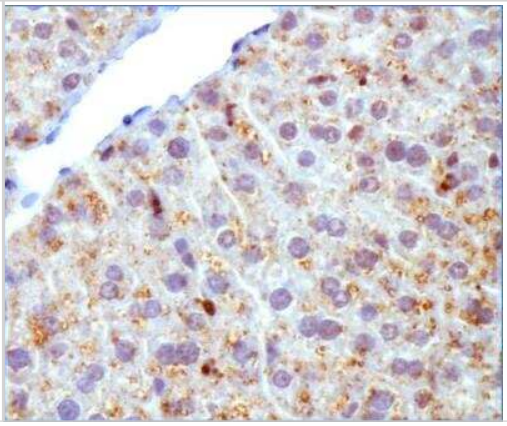
Perilipin-2/ADFP Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	51 kDa

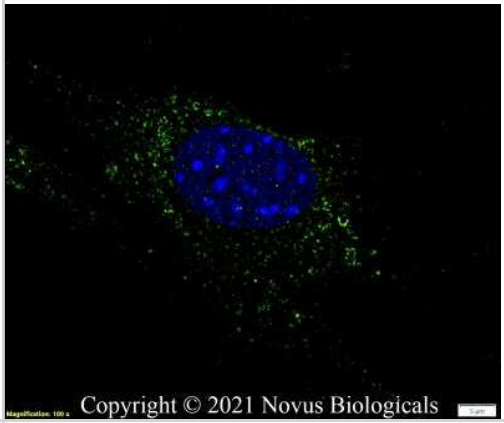
Product Description	
Host	Rabbit
Gene ID	123
Gene Symbol	PLIN2
Species	Human, Mouse, Rat, Bovine
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 29053516). Use in Bovine reported in scientific literature (PMID:32647143).
Immunogen	A synthetic peptide made to a C-terminal region of mouse ADFP (within residues 350-425) [Swiss-Prot# P43883]

Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Dual RNAscope ISH-IHC
Recommended Dilutions	Western Blot 2 ug/ml, Simple Western 1:50, Immunohistochemistry, Immunocytochemistry/ Immunofluorescence 1:50-1:200, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen reported in scientific literature (PMID 35041644), Dual RNAscope ISH-IHC
Application Notes	This ADFP antibody can be used for Western blot and Immunofluorescence/Immunocytochemistry. In Western blot a band is observed at approx. 51 kDa. In ICC/IF, membrane staining was observed in HeLa cells. In IHC-P, staining was observed on the membrane of mouse liver cells Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.

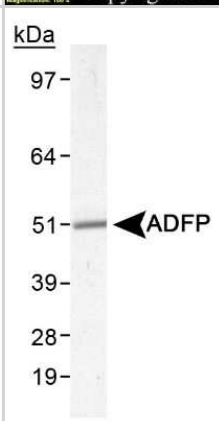
Images

Formalin-fixed paraffin-embedded tissue sections of mouse intestine were probed for Perilipin-2/ADFP mRNA (ACD RNAScope Probe, catalog #577111; Fast Red chromogen, ACD catalog # 322750). Adjacent tissue section was processed for immunohistochemistry using Rabbit Polyclonal (Novus Biologicals catalog # NB110-40877) at 1:150 dilution with overnight incubation at 4 degrees Celsius followed by incubation with anti-rabbit IgG VisUCyte HRP Polymer Antibody (Catalog # VC003) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to intestinal villi.	
Simple Western lane view shows a specific band for ADFP in 0.5 mg/ml of HepG2 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Oleic acid increasesI2 oxidation, glucose utilization and expression of genes involved in LCFA metabolism in U138 GBM cells. Cropped western blot images and quantitation of PLIN2 and GAPDH protein levels from cell lysates. Human hepatoma (VL17A) cell line was used as a positive control (+control). Full length blots are in Supplementary Fig. A1. N=3 independent experiments in triplicate. Image collected and cropped by Citeab from the following publication (Lipid accumulation and oxidation in glioblastoma multiforme. Sci Rep (2019)) licensed under a CC-BY license.	
ADFP antibody was tested in mouse liver using DAB with hematoxylin counterstain.	

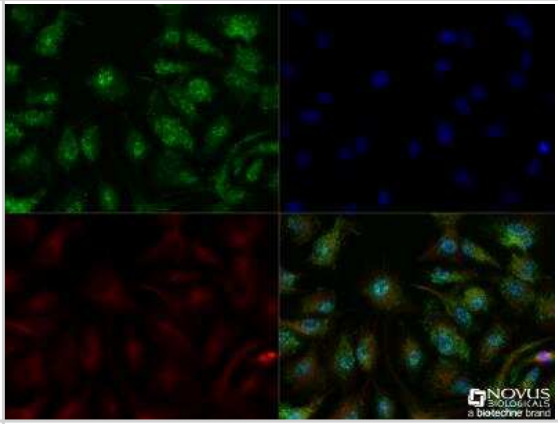
NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-Perilipin-2/ADFP Antibody NB110-40877 at 1 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



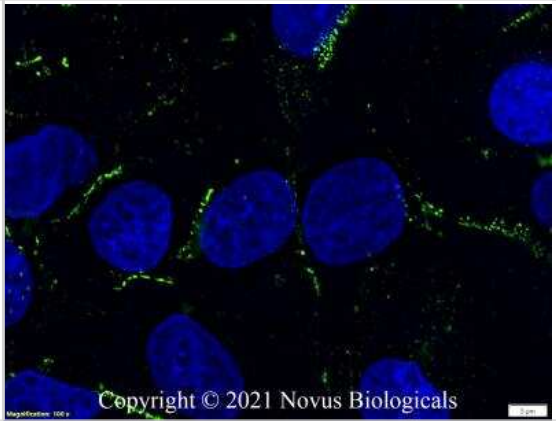
Detection of murine ADFP in mouse liver lysate.



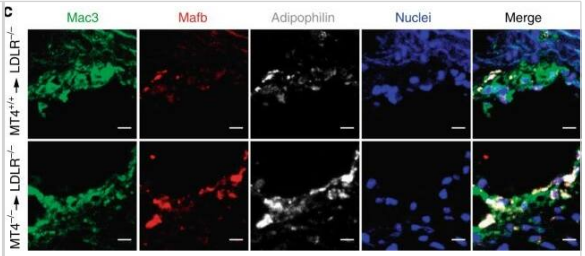
HepG2 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti-Perilipin-2/ADFP at a 1:100 dilution overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Alpha tubulin (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.



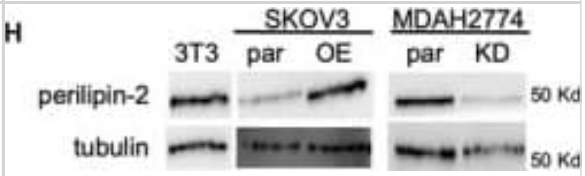
HepG2 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-Perilipin-2/ADFP Antibody NB110-40877 at 1 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



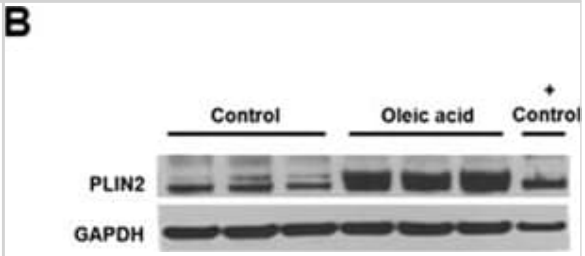
Lack of MT4-MMP in patrolling monocytes leads to the accumulation of Mafb+AIM+ macrophages in incipient atherosclerotic plaques. c) Representative images of transverse sections of aortic sinus from BM-transplanted Ldlr-/- mice fed a HFD for 1 week; sections were labeled for Mac3 (green), Mafb (red), and adipophilin (white), and with Hoechst (blue; nuclei); scale bar, 10 um. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41467-018-03351-4), licensed under a CC-BY licence.



Manipulation of LDLR or LPC abundance could reprogramme LD metabolism and increase DNA adduct formation for enhancing the cisplatin cytotoxic effects. H): Perilipin-2, which is an LD marker protein, was detected in OE and KD LDLR in SKOV3 and MDAH-2774 cells, respectively. The expression of 3T3 cells served as a positive control of perilipin-2 expression. The tubulin expression served as a loading control for LD. Here, * or #P < .05 and ** or ##P < .01, based on the t test results from at least three reproducible experiments Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32543783), licensed under a CC-BY licence.



Oleic acid increases beta oxidation, glucose utilization and expression of genes involved in LCFA metabolism in U138 GBM cells. (B) Cropped western blot images and quantitation of PLIN2 and GAPDH protein levels from cell lysates. Human hepatoma (VL17A) cell line was used as a positive control (+control). Full length blots are in Supplementary Fig. 1. N = 3 independent experiments in triplicate. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31863022), licensed under a CC-BY licence.



Publications

Renier TJ, Paetz OR, Paal MC et al. Changing the phospholipid composition of lipid droplets alters localization of select lipid droplet proteins microPublication biology 2023-11-03 [PMID: 38021172] (SDS-Page, Mouse)

Kothari V, Savard C, Tang J et al. sTREM2 is a plasma biomarker for human NASH and promotes hepatocyte lipid accumulation Hepatology communications 2023-11-01 [PMID: 37820278] (ICC/IF, Mouse)

Wieland EB, Kempen LJAP, Lu C et al. Protocol for multispectral imaging on cryosections to map myeloid cell heterogeneity in its spatial context STAR protocols 2023-09-23 [PMID: 37742177] (IHC-Fr)

Details:
Alexa Fluor 488 conjugation used. Diltion 1:29

Plewes MR, Krause C, Talbott HA et al. Trafficking of cholesterol from lipid droplets to mitochondria in bovine luteal cells: Acute control of progesterone synthesis The FASEB Journal 2020-08-01 [PMID: 32614098] (WB)

Wang CH, Liu HM, Chang ZY et al. Losartan Prevents Hepatic Steatosis and Macrophage Polarization by Inhibiting HIF-1? in a Murine Model of NAFLD International Journal of Molecular Sciences 2021-07-22 [PMID: 34360607]

Mi?i? B, Djordjevic A, Veli?kovi? N et al. AMPK Activation as a Protective Mechanism to Restrain Oxidative Stress in the Insulin-Resistant State in Skeletal Muscle of Rat Model of PCOS Subjected to Postnatal Overfeeding Biomedicines 2023-05-30 [PMID: 37371678]

Chavanet A, Hill KR, Jim□nez-Andrade Y et al. Intracellular signaling modules linking DNA damage to secretome changes in senescent melanoma cells Melanoma Research 2020-08-01 [PMID: 32628430] (ICC/IF, WB)

Pratelli G, Di Liberto D, Carlisi D et al. Hypertrophy and ER Stress Induced by Palmitate Are Counteracted by Mango Peel and Seed Extracts in 3T3-L1 Adipocytes International journal of molecular sciences 2023-03-12 [PMID: 36982490] (WB, Plant)

Choi YJ, Yun SH, Yu J et al. Chaperone-mediated autophagy dysregulation during aging impairs hepatic fatty acid oxidation via accumulation of NCoR1 Molecular metabolism 2023-07-29 [PMID: 37524243] (WB, Mouse)

Androvic P, Schifferer M, Perez Anderson K et al. Spatial Transcriptomics-correlated Electron Microscopy maps transcriptional and ultrastructural responses to brain injury Nature communications 2023-07-11 [PMID: 37433806] (IHC, Mouse)

Ibayashi M, Aizawa R, Mitsui J, Tsukamoto S Lipid droplet synthesis is associated with angiogenesis in mouse ovarian follicles Biology of reproduction 2022-12-28 [PMID: 36579469]

Wang N, Wang M, Jeevaratnam S et al. Opposing effects of apoE2 and apoE4 on microglial activation and lipid metabolism in response to demyelination Molecular neurodegeneration 2022-11-23 [PMID: 36419137] (IF/IHC, Mouse)

More publications at http://www.novusbio.com/NB110-40877

Procedures

Western Blot protocol specific for ADFP Antibody (NB110-40877)

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%.

Immunocytochemistry/Immunofluorescence Protocol for ADFP Antibody (NB110-40877)

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.

Immunohistochemistry-Paraffin protocol for Perilipin-2/ADFP Antibody (NB110-40877)

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 4 degrees C.
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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Products Related to NB110-40877

NB820-59232	Human Liver Whole Tissue Lysate (Adult Whole Normal)
NB110-40877PEP	Perilipin-2/ADFP 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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