Product Datasheet

Perilipin-2/ADFP Antibody
NB110-40877

Unit Size: 0.1 ml
Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

Publications: 12
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Updated 1/30/2018 v.20.1

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**Product Information**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
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<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
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<tr>
<td>Concentration</td>
<td>1.17 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Aliquot and store at -20°C or -80°C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.05% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td>Buffer</td>
<td>PBS and 30% Glycerol</td>
</tr>
<tr>
<td>Target Molecular Weight</td>
<td>51 kDa</td>
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**Product Description**

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<thead>
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<th>Details</th>
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<tbody>
<tr>
<td>Host</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Gene ID</td>
<td>123</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>PLIN2</td>
</tr>
<tr>
<td>Species</td>
<td>Human, Mouse, Rat</td>
</tr>
<tr>
<td>Reactivity Notes</td>
<td>Customer feedback has been negative on trout liver. Rat reactivity reported in scientific literature (PMID: 29053516).</td>
</tr>
<tr>
<td>Immunogen</td>
<td>A synthetic peptide made to a C-terminal region of mouse ADFP (within residues 350-425) [Swiss-Prot # P43883]</td>
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**Product Application Details**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
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<tbody>
<tr>
<td>Applications</td>
<td>Western Blot, Simple Western, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin</td>
</tr>
<tr>
<td>Recommended Dilutions</td>
<td>Western Blot 2 ug/ml, Simple Western 1:50, Immunohistochemistry, Immunocytochemistry/Immunofluorescence 1:50-1:200, Immunohistochemistry-Paraffin 1:200</td>
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<tr>
<td>Application Notes</td>
<td>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. 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.</td>
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Immunocytochemistry/Immunofluorescence: Perilipin-2/ADFP Antibody [NB110-40877] - 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.

Immunohistochemistry: Perilipin-2/ADFP Antibody [NB110-40877] - ADFP antibody was tested in mouse liver using DAB with hematoxylin counterstain.

Immunocytochemistry/Immunofluorescence: Perilipin-2/ADFP Antibody [NB110-40877] - Detection of ADFP (Green) in Hela cells using NB110-40877 at a 1:50 dilution. Nuclei (Blue) were counterstained using Hoechst 33258.
Simple Western: Perilipin-2/ADFP Antibody [NB110-40877] - 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.

<table>
<thead>
<tr>
<th>Publications</th>
<th>Details:</th>
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<tbody>
<tr>
<td>Blossom SJ, Fernandes L, Bai S et al. Opposing actions of developmental trichloroethylene and high-fat-diet co-exposure on markers of lipogenesis and inflammation in autoimmune-prone mice. Toxicol. Sci. Apr 12 2018 12:00AM [PMID: 29669109] (IHC, Mouse)</td>
<td></td>
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<td>Covington JD, Johansson DL, Coen PM et al. Intramyocellular Lipid Droplet Size Rather Than Total Lipid Content is Related to Insulin Sensitivity After 8 Weeks of Overfeeding Obesity (Silver Spring). 2017 Dec 01 [PMID: 29071793] (Human)</td>
<td></td>
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<tr>
<td>Nishikawa K, Iwaya K, Kinoshita M et al. Resveratrol increases CD68(+) Kupffer cells co-localized with adipose differentiation-related protein (ADFP) and ameliorates high-fat-diet-induced fatty liver in mice Mol Nutr Food Res. 2015 Feb 12 [PMID: 25677089] (WB, Mouse)</td>
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Details:
Perilipin-2/ADFP antibody (0.5 ug/ml) used for WB on mouse liver extract (Fig. 4).


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 wash sections in washing solution for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 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 Embedded Sections (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. Wash sections in wash solution for 10 minutes. Add H2O2 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash three times for 10 minutes.
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.
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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