Product Datasheet

Adiponectin/Acrp30 Antibody
NB100-65810

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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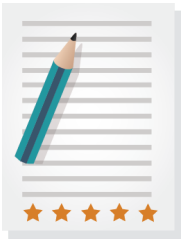
Updated 12/20/2023 v.20.1



NB100-65810

Adiponectin/Acrp30 Antibody

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	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description

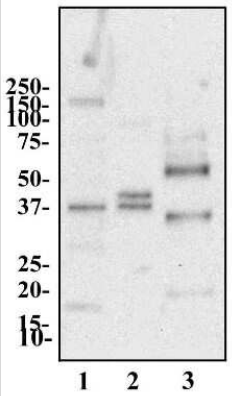
Host	Rabbit
Gene ID	9370
Gene Symbol	ADIPOQ
Species	Human, Mouse, Rat
Specificity/Sensitivity	This recognises an epitope within the C-terminal region (CT) of the 244 amino acid protein Adiponectin.
Immunogen	A synthetic peptide made to a C-terminal sequence of human Adiponectin (between amino acids 200-244) [UniProt Q15848]

Product Application Details

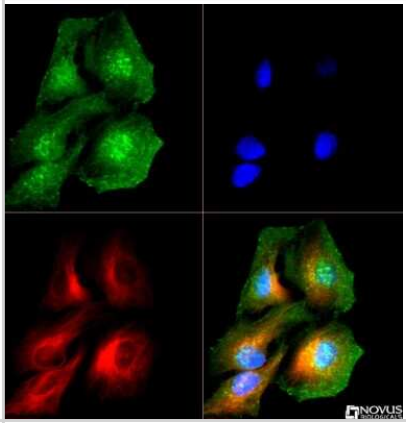
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1-2 ug/ml, Flow Cytometry 1:10-1:1000, Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence 1:10-1:500, Immunohistochemistry-Paraffin 10 ug/ml, Flow (Intracellular)
Application Notes	The theoretical molecular weight of Adiponectin is 27, however this protein is highly glycosylated.

Images

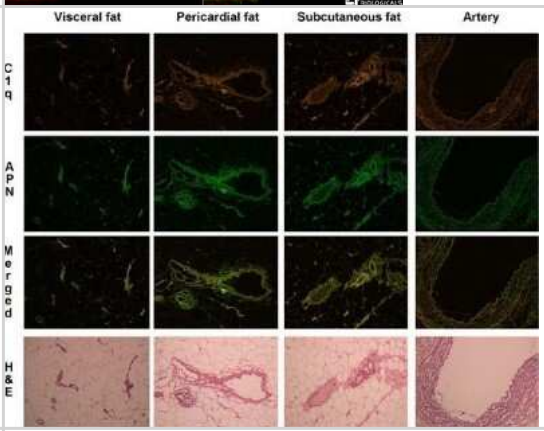
Western Blot: Adiponectin/Acrp30 Antibody [NB100-65810] - Western blot analysis of Adoponectin in 1. human liver 2. rat liver 3. HL60



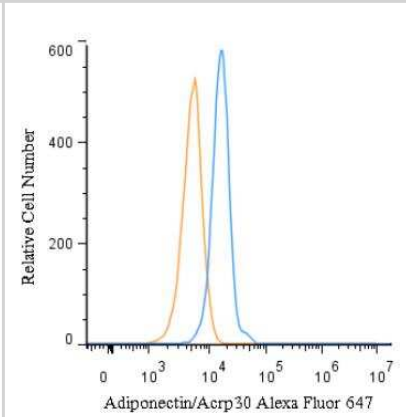
Immunocytochemistry/Immunofluorescence: Adiponectin/Acrp30 Antibody [NB100-65810] - Adiponectin antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red). Cytoplasmic, membrane and internal organelle staining was observed.



Immunohistochemistry-Paraffin: Adiponectin/Acrp30 Antibody [NB100-65810] - Co-localization of C1q and APN deposited in adipose tissue and blood vessels (x100). Positive staining of C1q (red) and APN (green) is shown along the perivascular areas of fat tissues and intimal-medial layer of the blood vessel. C1q and APN co-localized almost completely in the same areas. H&E, hematoxylin and eosin. Image collected and cropped by CiteAb from the following publication (https://www.cardiab.com/content/14/1/50), licensed under a CC-BY license.

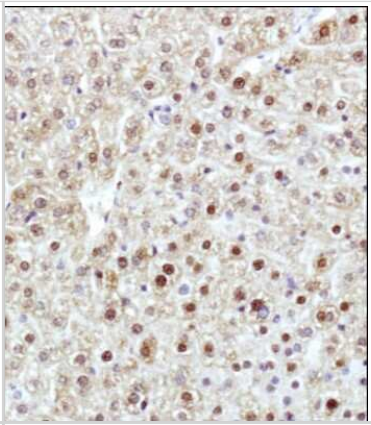


Flow (Intracellular): Adiponectin/Acrp30 Antibody [NB100-65810] - An intracellular stain was performed on Jurkat cells with Adiponectin/Acrp30 Antibody NB100-65810AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to AF647.

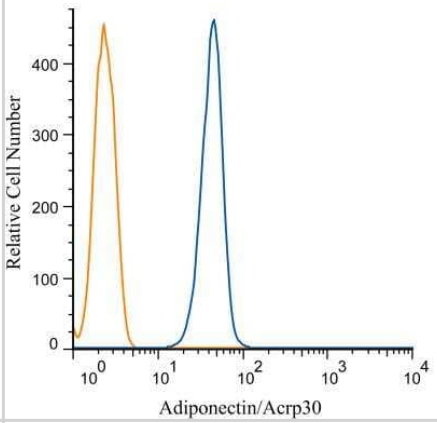


Immunocytochemistry/Immunofluorescence: Adiponectin/Acrp30 Antibody [NB100-65810] - Immunocytochemical staining of HL60 cells with Rabbit anti Human adiponectin.

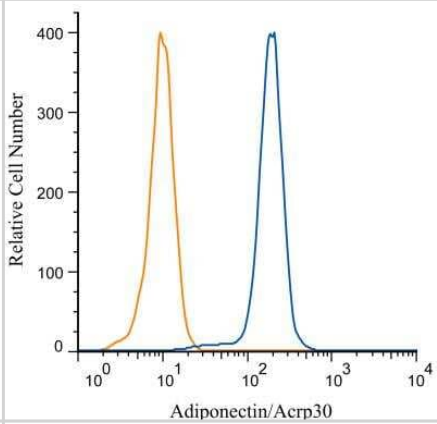
Immunohistochemistry-Paraffin: Adiponectin/Acrp30 Antibody [NB100-65810] - IHC analysis of Adiponectin in mouse liver showing nuclear and cytoplasmic staining.



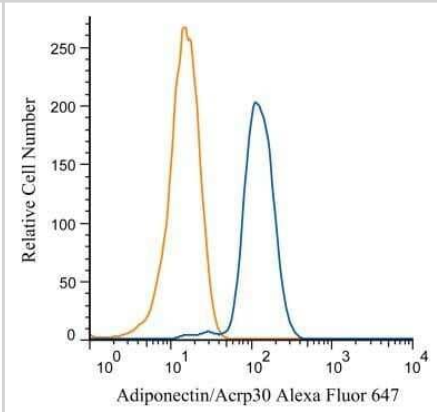
Flow Cytometry: Adiponectin/Acrp30 Antibody [NB100-65810] - An intracellular stain was performed on Jurkat cells with Adiponectin antibody NB100-65810 (blue) and a matched isotype control NBP2-24893 (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by Dylight488-conjugated anti-rabbit secondary antibody.

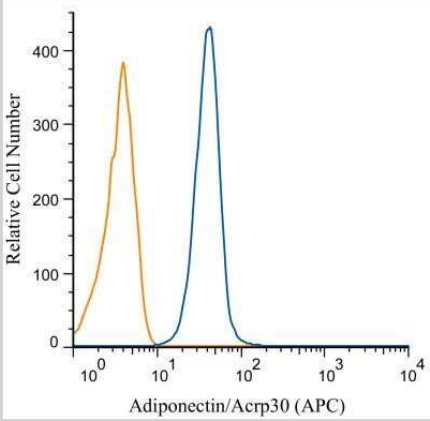
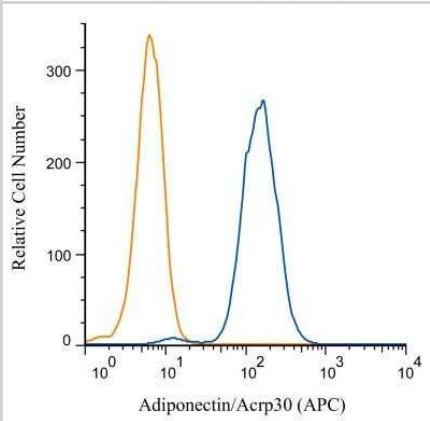


Flow Cytometry: Adiponectin/Acrp30 Antibody [NB100-65810] - An intracellular stain was performed on Raji cells with Adiponectin antibody NB100-65810 (blue) and a matched isotype control NBP2-24893 (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by Dylight488-conjugated anti-rabbit secondary antibody.



Flow Cytometry: Adiponectin/Acrp30 Antibody [NB100-65810] - Analysis of Alexa Fluor 647 conjugate of NB100-65810. An intracellular stain was performed on Jurkat cells with Adiponectin antibody NB100-65810AF647 (blue) and a matched isotype control NBP2-24893AF647 (orange). Cells were fixed with 4% PFA and then perm



Flow Cytometry: Adiponectin/Acrp30 Antibody [NB100-65810] - Analysis of Allophycocyanin conjugate of NB100-65810. An intracellular stain was performed on Jurkat cells with Adiponectin antibody NB100-65810APC (blue) and a matched isotype control NBP2-24893APC (orange). Cells were fixed with 4% PFA and then permeabl	
Flow Cytometry: Adiponectin/Acrp30 Antibody [NB100-65810] - Analysis of Allophycocyanin conjugate of NB100-65810. An intracellular stain was performed on Raji cells with Adiponectin antibody NB100-65810APC (blue) and a matched isotype control NBP2-24893APC (orange). Cells were fixed with 4% PFA and then permeabliz	

Publications

Conti G, Calderan L, Quintero Sierra LA et al. Tumor and peritumoral adipose tissue crosstalk: de-differentiated adipocytes influence spread of colon carcinoma cells Tissue and Cell 2022-12-01 [PMID: 36542947] (WB, Mouse)

Cho J, Teshigawara R, Kameda M et al. Nucleus-localized adiponectin is survival gate keeper through miR-214-mediated AIFM2 regulation Genes Cells 2018-11-25 [PMID: 30474186] (ICC/IF, Mouse)

Waragai M, Adame A, Trinh I et al. Possible Involvement of Adiponectin, the Anti-Diabetes Molecule, in the Pathogenesis of Alzheimer's Disease. J. Alzheimers Dis. 2016-04-08 [PMID: 27079710] (IHC-P, Human)

Hong ES, Lim C, Choi HY et al. The amount of C1q-adiponectin complex is higher in the serum and the complex localizes to perivascular areas of fat tissues and the intimal-medial layer of blood vessels of coronary artery disease patients. Cardiovasc Diabetol. 2015-05-14 [PMID: 25956582] (IHC-P, Human)

Details:
Adiponectin/Acrp30 antibody was used for IHC-P analysis of adipose/fat tissues (subcutaneous, visceral, and pericardial areas) and internal mammary artery sections from healthy controls and patients with coronary artery disease. The IHC-P assay implicated 4% paraformaldehyde fixation, ON 4C incubation of primary antibody and signal detection using Alexa Fluor 488 goat anti-rabbit IgG secondary antibody. Adiponectin/Acrp30 (APN) was found to be co-localized with complement component C1q along the perivascular areas of fat tissues and intimal–medial layer of the blood vessel (Figure 2).

Canhoroz M, Kanat O, Saraydaroglu O et al. Clinical significance of adiponectin expression in colon cancer patients. J Cancer Res Ther 2014-07-15 [PMID: 25022390] (IHC-P, Human)

Details:
Adiponectin antibody used for IHC-P on human colon cancer patients. Primary diluted at 1:100 and incubated for 1 hour followed by detection with biolynated link reagent-DAB detection. Staining images not shown but see the full text for detailed IHC-P protocol.

Trayhurn, P Wood, IS. Signalling role of adipose tissue: adipokines and inflammation in obesity. Biochem Soc Trans 33: 1078-1081. 2005-01-01 [PMID: 16246049]

Lihn, AS et al. Adiponectin: action, regulation and association to insulin sensitivity. Obes Rev 6: 13-21. 2005-01-01 [PMID: 15655035]

Wong, GW et al. A family of Acrp30/adiponectin structural functional paralogs. P.N.A.S. 101: 10302-10307. P.N.A.S. 101: 10302-10307. 2004-01-01 [PMID: 15231994] (IHC-P)

Procedures

Western Blot Protocol for Adiponectin Antibody (NB100-65810)
Adiponectin/Acrp30 Antibody:
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-HDAC5 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%.

Immunohistochemistry-Paraffin Protocol for Adiponectin Antibody (NB100-65810)
Adiponectin/Acrp30 Antibody:
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 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.

Immunocytochemistry/Immunofluorescence Protocol for Adiponectin Antibody (NB100-65810)
Adiponectin/Acrp30 Antibody:
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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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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