# abcam

### Product datasheet

## Anti-Adiponectin antibody [19F1] ab22554

\*\*\*\*\* 16 Abreviews 89 References 8 Images

#### Overview

Product name	Anti-Adiponectin antibody [19F1]	
FIGUELHame		
Description	Mouse monoclonal [19F1] to Adiponectin	
Host species	Mouse	
Tested applications	Suitable for: ICC, IHC-P, WB	
Species reactivity	Reacts with: Mouse, Human	
Immunogen	Recombinant full length protein (Human).	
Positive control	3T3-L1 adipocytes	
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.	
	If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As	

#### **Properties**

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	Preservative: 0.05% Sodium azide Constituents: PBS, 0.1% BSA
Purity	Protein G purified
Clonality	Monoclonal
Clone number	19F1
lsotype	lgG

#### Applications

The Abpromise guarantee

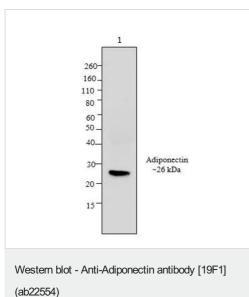
Our Abpromise guarantee covers the use of ab22554 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC		Use a concentration of 2 µg/ml.
IHC-P	★ ★ ★ ★ ★ <u>(5)</u>	Use at an assay dependent concentration.
WB	<b>★ ★ ★ ★</b> ★ ★ ★ <u>(10)</u>	Use a concentration of 2 µg/ml. Detects a band of approximately 30 kDa (predicted molecular weight: 26 kDa).

#### Target

Function	Important adipokine involved in the control of fat metabolism and insulin sensitivity, with direct anti- diabetic, anti-atherogenic and anti-inflammatory activities. Stimulates AMPK phosphorylation and activation in the liver and the skeletal muscle, enhancing glucose utilization and fatty-acid combustion. Antagonizes TNF-alpha by negatively regulating its expression in various tissues such as liver and macrophages, and also by counteracting its effects. Inhibits endothelial NF- kappa-B signaling through a cAMP-dependent pathway. May play a role in cell growth, angiogenesis and tissue remodeling by binding and sequestering various growth factors with distinct binding affinities, depending on the type of complex, LMW, MMW or HMW.
Tissue specificity	Synthesized exclusively by adipocytes and secreted into plasma.
Involvement in disease	Defects in ADIPOQ are the cause of adiponectin deficiency (ADPND) [MIM:612556]. ADPND results in very low concentrations of plasma adiponectin. Genetic variations in ADIPOQ are associated with non-insulin-dependent diabetes mellitus (NIDDM) [MIM:125853]; also known as diabetes mellitus type 2. NIDDM is characterized by an autosomal dominant mode of inheritance, onset during adulthood and insulin resistance.
Sequence similarities	Contains 1 C1q domain. Contains 1 collagen-like domain.
Domain	The C1q domain is commonly called the globular domain.
Post-translational modifications	<ul> <li>Hydroxylated Lys-33 was not identified in PubMed:16497731, probably due to poor representation of the N-terminal peptide in mass fingerprinting.</li> <li>HMW complexes are more extensively glycosylated than smaller oligomers. Hydroxylation and glycosylation of the lysine residues within the collagene-like domain of adiponectin seem to be critically involved in regulating the formation and/or secretion of HMW complexes and consequently contribute to the insulin-sensitizing activity of adiponectin in hepatocytes.</li> <li>O-glycosylated. Not N-glycosylated. O-linked glycans on hydroxylysines consist of Glc-Gal disaccharides bound to the oxygen atom of post-translationally added hydroxyl groups. Sialylated to varying degrees depending on tissue. Thr-22 appears to be the major site of sialylation. Higher sialylation found in SGBS adipocytes than in HEK fibroblasts. Sialylated hydroxylysines.</li> <li>Desialylated forms are rapidly cleared from the circulation.</li> </ul>
Cellular localization	Secreted.



Anti-Adiponectin antibody [19F1] (ab22554) at 2  $\mu g/ml$  + Hep G2 cell line

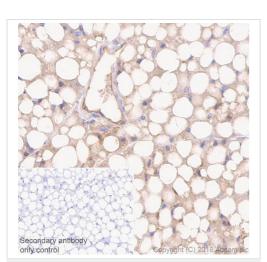
#### Secondary

Goat anti-Mouse lgG (H+L) Superclonal<sup>™</sup> Secondary Antibody, HRP at 1/2500 dilution

Predicted band size: 26 kDa

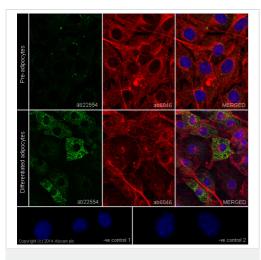
A ~26 kDa band corresponding Adiponectin was observed in the cell line tested.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) of Mouse Adipose tissue staining Adiponectin using

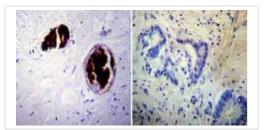


ab22554 (PBS in negative control) at 1:200 (5µg/ml). ImmunoHistoProbe one step HRP Polymer (ready to use) used. Performed heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0)

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Adiponectin antibody [19F1] (ab22554)



Immunocytochemistry - Anti-Adiponectin antibody [19F1] (ab22554)

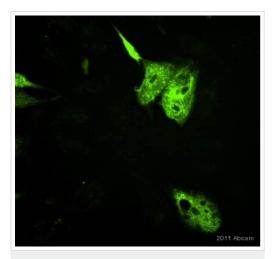


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Adiponectin antibody [19F1] (ab22554)

ab22554 staining Adiponectin in 3T3-L1 cells (ATCC® CL-173 TM). Increased expression of Adiponectin correlates with adipocyte phenotype, as described in literature. Cells, grown to confluency in DMEM with 10% FBS, were differentiated by stimulation for two days with 0.5 mM 3-isobutyl-1-methylxanthine (ab120840), 0.25uM dexamethasone (ab120743) and 1ug/ml insulin (ab123768), followed by two more days with only insulin. Cells were maintained for an additional three days in growth medium alone. Undifferentiated and differentiated adipocytes were fixed with 100% methanol (5min) at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 1h at room temperature. Staining of the treated cells with ab22554 (2.5µg/ml) and ab6046 at 1µg/ml overnight at +4°C, was followed by a further incubation at room temperature for 1h with an AlexaFluor®488 Goat anti-Rabbit secondary (ab150081) at 2 µg/ml (shown in green) and AlexaFluor®594 Goat anti-Mouse secondary (ab150120) at 2 µg/ml (shown in pseudo colour red). Nuclear DNA was labelled in blue with DAPI. Negative controls: 1- Rabbit primary and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.

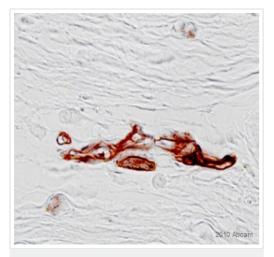
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Immunohistochemistry was performed on cancer biopsies of deparaffinized Human colon carcinoma tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:100 with a mouse monoclonal antibody recognizing Adiponectin ab22554 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.

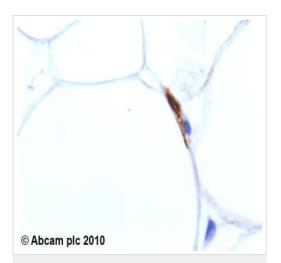


ab22554 staining Adiponectin in human adipose stem cells by Immunocytochemistry/ Immunofluorescence. The cells were fixed in paraformaldehyde, permeabilised in 0.1% Triton X and then blocked using 4% serum for 1 hour. Samples were then incubated with primary antibody at 1/500 for 1 hour 30 minutes. The secondary antibody used was a goat IgG conjugated to Alexa Fluor® 488 (green) used at a 1/500 dilution.

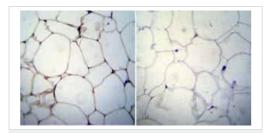
Immunocytochemistry - Anti-Adiponectin antibody [19F1] (ab22554) Image courtesy of an anonymous Abreview.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Adiponectin antibody [19F1] (ab22554) This image is courtesy of an anonymous Abreview ab22554 staining Adiponectin in Mouse skin tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Tissue was fixed with paraformaldehyde and blocked with 10% serum for 1 hour at 20°C; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody (1/100) for 12 hours at 4°C. A Biotinconjugated Rabbit anti-mouse polyclonal (1/200) was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Adiponectin antibody [19F1] (ab22554)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Adiponectin antibody [19F1] (ab22554) ab22554 (4µg/ml) staining adiponectin in human breast, using an automated system (DAKO Autostainer Plus). Using this protocol there is nuclear and cytoplasmic staining. Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer EDTA pH 9.0 in a DAKO PT link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.

Immunohistochemistry was performed on biopsies of deparaffinized Human skin tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:20 with a mouse monoclonal antibody recognizing Adiponectin ab22554 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.

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