abcam

Product datasheet

Anti-SCD1 antibody [CD.E10] ab19862

KO VALIDATED

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Overview

Product name	Anti-SCD1 antibody [CD.E10]
Description	Mouse monoclonal [CD.E10] to SCD1
Host species	Mouse
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, Flow Cyt, IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant full length protein (Human).
Positive control	WB: HepG2 and HeLa cell lysates. Flow Cyt: HepG2 cells. IP: Hek293 whole cell extract. IHC: Human skin tissue.
General notes	SCD1 is known to undergo post-translational modifications and the sizes differ in different cell lines so the observed band size can be different than predicted band size. As positive control we recommend using SCD1 over-expressed 293 transfected cell lysates for western blot. This product was changed from ascites to tissue culture supernatant on 25th May 2018. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team. 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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.05% Sodium azide Constituent: PBS

Purity	Protein A purified
Purification notes	Protein A affinity chromatography
Clonality	Monoclonal
Clone number	CD.E10
Isotype	IgG2b

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab19862 in the following tested applications.

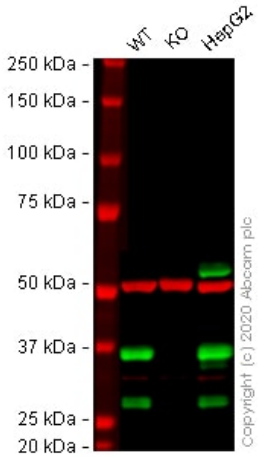
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆ (3)	1/1000. Predicted molecular weight: 42 kDa.
IHC-P	★☆☆☆☆ (1)	Use a concentration of 4 µg/ml.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt	★★★★☆ (2)	Use 1µg for 10^6 cells. **ab170192** - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration.

Target

Function	Terminal component of the liver microsomal stearyl-CoA desaturase system, that utilizes O(2) and electrons from reduced cytochrome b5 to catalyze the insertion of a double bond into a spectrum of fatty acyl-CoA substrates including palmitoyl-CoA and stearoyl-CoA.
Sequence similarities	Belongs to the fatty acid desaturase family.
Domain	The histidine box domains may contain the active site and/or be involved in metal ion binding.
Cellular localization	Endoplasmic reticulum membrane.

Images



Western blot - Anti-SCD1 antibody [CD.E10] (ab19862)

All lanes : Anti-SCD1 antibody [CD.E10] (ab19862) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : SCD knockout HeLa cell lysate
Lane 3 : HepG2 cell lysate

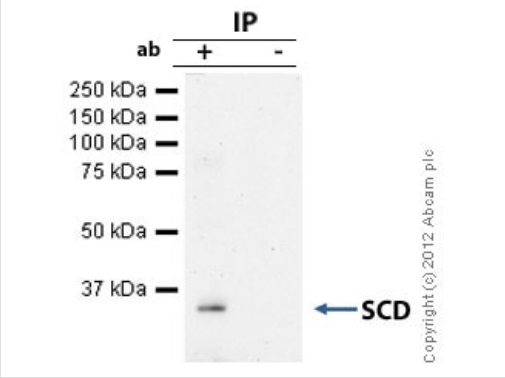
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 42 kDa
Observed band size: 36 kDa

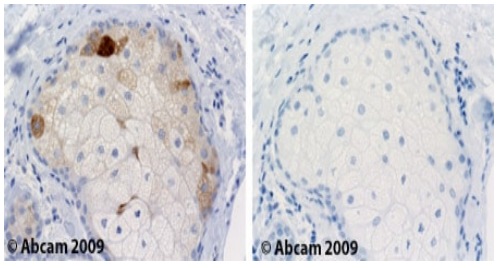
Lanes 1 - 4: Merged signal (red and green). Green - ab19862 observed at 36 kDa. Red - loading control **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55kDa.

ab19862 was shown to react with SCD1 in wild-type HeLa cells in western blot with loss of signal observed in SCD knockout cell line **ab265220** (SCD knockout cell lysate **ab257658**). Wild-type and SCD knockout HeLa cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab19862 and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



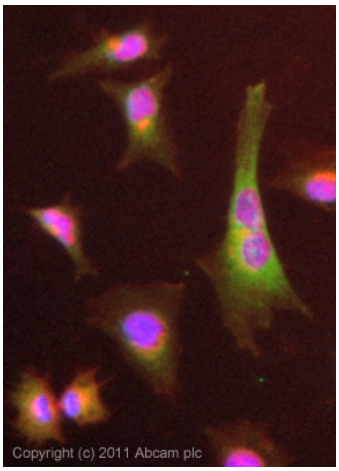
Immunoprecipitation - Anti-SCD1 antibody [CD.E10] (ab19862)

SCD was immunoprecipitated using 0.5mg Hek293 whole cell extract, 10µg of Mouse monoclonal to SCD and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, Hek293 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab19862.
Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/5000 dilution.
Band: 32kDa; SCD.



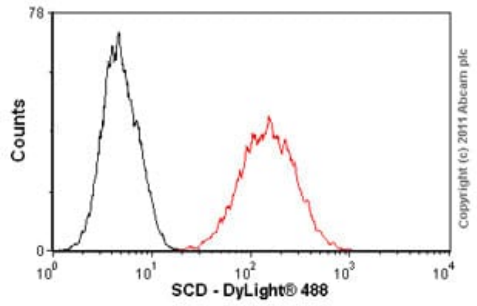
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SCD1 antibody [CD.E10] (ab19862)

ab19862 staining SCD in human skin.
Left panel: with primary antibody at 4 ug/ml. Right panel: isotype control.
Sections were stained using an automated system (DAKO Autostainer Plus), at room temperature: sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffers EDTA pH 9.0. 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 we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.



Immunocytochemistry/ Immunofluorescence - Anti-SCD1 antibody [CD.E10] (ab19862)

ICC/IF image of ab19862 stained HeLa cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab19862, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Flow Cytometry - Anti-SCD1 antibody [CD.E10] (ab19862)

Overlay histogram showing HepG2 cells stained with ab19862 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab19862, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (**ab91366**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HepG2 cells fixed with 80% methanol (5 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

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