abcam

Product datasheet

Anti-Sortilin/NT3 antibody ab16640



**** 10 Abreviews 88 References 4 Images

Overview

Product name Anti-Sortilin/NT3 antibody

Description Rabbit polyclonal to Sortilin/NT3

Host species Rabbit

Tested applications Suitable for: IHC-FoFr, WB

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Cow

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa cell lysate; NIH/3T3 cell lysate; 293T cell lysate; Human, Mouse, and Rat Brain tissue

lysates. IHC-FoFr: Rat cortical neurons.

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. 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: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

scientific support team who will be happy to help.

Purity Immunogen affinity purified

1

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab16640 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
IHC-FoFr	*** <u>*</u> (2)	1/3000.
WB	★★★★☆ (5)	Use a concentration of 1 µg/ml. Detects a band of approximately 100 kDa (predicted molecular weight: 95 kDa).

Target

Function

Functions as a sorting receptor in the Golgi compartment and as a clearance receptor on the cell surface. Required for protein transport from the Golgi apparatus to the lysosomes by a pathway that is independent of the mannose-6-phosphate receptor (M6PR). Also required for protein transport from the Golgi apparatus to the endosomes. Promotes neuronal apoptosis by mediating endocytosis of the proapoptotic precursor forms of BDNF (proBDNF) and NGFB (proNGFB). Also acts as a receptor for neurotensin. May promote mineralization of the extracellular matrix during osteogenic differentiation by scavenging extracellular LPL. Probably required in adipocytes for the formation of specialized storage vesicles containing the glucose transporter SLC2A4/GLUT4 (GLUT4 storage vesicles, or GSVs). These vesicles provide a stable pool of SLC2A4 and confer increased responsiveness to insulin. May also mediate transport from the endoplasmic reticulum to the Golgi.

Tissue specificity

Expressed at high levels in brain, spinal cord, heart, skeletal muscle, thyroid, placenta and testis. Expressed at lower levels in lymphoid organs, kidney, colon and liver.

Involvement in disease

Note=A common polymorphism located in a non-coding region between CELSR2 and PSRC1 alters a CEBP transcription factor binding site and is responsible for changes in hepatic expression of SORT1. Altered SORT1 expression in liver affects low density lipoprotein cholesterol levels in plasma and is associated with susceptibility to myocardial infarction.

Sequence similarities

Belongs to the VPS10-related sortilin family. SORT1 subfamily.

Contains 9 BNR repeats.

Domain

The N-terminal propeptide may facilitate precursor transport within the Golgi stack. Intrachain binding of the N-terminal propeptide and the extracellular domain may also inhibit premature

ligand binding.

The extracellular domain may be shed following protease cleavage in some cell types.

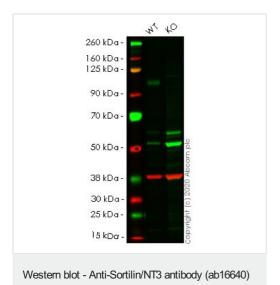
Post-translational modifications

The N-terminal propeptide is cleaved by furin and possibly other homologous proteases.

Cellular localization

Membrane. Endoplasmic reticulum membrane. Endosome membrane. Golgi apparatus > Golgi stack membrane. Lysosome membrane. Nucleus membrane. Cell membrane. Lysosome membrane. Localized to membranes of the endoplasmic reticulum, endosomes, Golgi stack, lysosomes and nucleus. A small fraction of the protein is also localized to the plasma membrane. May also be found in SLC2A4/GLUT4 storage vesicles (GSVs) in adipocytes. Localization to the

Images



All lanes: Anti-Sortilin/NT3 antibody (ab16640) at 1 µg/ml

Lane 1: Wild-type HeLa cell lysate

Lane 2: SORT1 knockout HeLa cell lysate

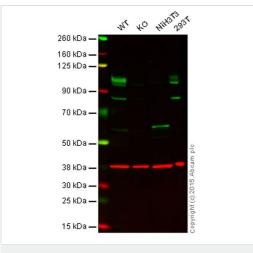
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

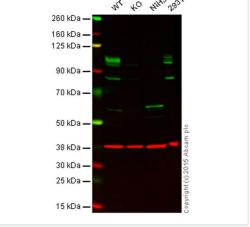
Predicted band size: 95 kDa **Observed band size:** 100 kDa

Lanes 1-2: Merged signal (red and green). Green - ab16640 observed at 100 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab16640 was shown to react with Sortilin/NT3 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab264772 (knockout cell lysate ab257696) was used. Wild-type HeLa and SORT1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab16640 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at a 1 µg/ml and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Sortilin/NT3 antibody (ab16640)





Western blot - Anti-Sortilin/NT3 antibody (ab16640)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: Sortilin/NT3 knockout HAP1 cell lysate (20 µg)

Lane 3: NIH/3T3 cell lysate (20 µg)

Lane 4: 293T cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab16440 observed at 100 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab16640 was shown to specifically react with Sortilin/NT3 in wildtype HAP1 cells. No band was observed when Sortilin/NT3 knockout samples were examined. Wild-type and Sortilin/NT3 knockout samples were subjected to SDS-PAGE. ab16640 and ab8245 (loading control to GAPDH) were diluted 1 µg/mL and 1/10,000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit lqG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.

Lane 1: Marker

Lanes 2-4: Anti-Sortilin/NT3 antibody (ab16640) at 1 µg/ml

Lane 2: Human Brain tissue lysate at 20 µg

Lane 3: Mouse Brain tissue lysate at 20 µg

Lane 4: Rat Brain whole cell lysate at 20 µg

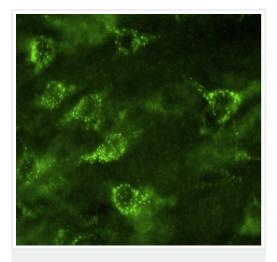
Secondary

Lanes 2-4: Goat polyclonal to Rabbit IgG H&L (HRP) Pre-Adsorbed at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 95 kDa

Additional bands at: 31 kDa (possible cleavage fragment)



Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-Sortilin/NT3 antibody (ab16640)

This image is courtesy of Sophie Pezet, Univ London Kings Coll, United Kingdom

Immunofluorescent staining for Sortilin/NT3 in rat cortical neurons using ab16640. The staining is cytoplasmic and punctate of many cells such as cortical neurons. The image was taken with a X20 objective.

Protocol details: Rats were intracardially perfused with 4% paraformaldehyde. Whole brain tissue was post-fixed overnight in the same fixative, cryoprotected in 20% sucrose and frozen in OCT and then cut on cryostat (30µm coronal sections). IHC was perfored in free floating with fixed tissues (rat brain sections). Primary antibody was incubated overnight at 1/3000 at room temperature

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