abcam

Product datasheet

Anti-EBP50/NHERF-1 antibody [EPR5562] ab109430

KO VALIDATED Recombinant RabMAb

2 References 6 Images

Overview

Product name	Anti-EBP50/NHERF-1 antibody [EPR5562]
Description	Rabbit monoclonal [EPR5562] to EBP50/NHERF-1
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P **Unsuitable for:** Flow Cyt,ICC/IF or IP
Species reactivity	**Reacts with:** Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	HCT116, HepG2, 293T, Jurkat, C6, PC-12 and MCF-7 cell lysates; Human kidney tissue
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Mouse: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR5562
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab109430 in the following tested applications.

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

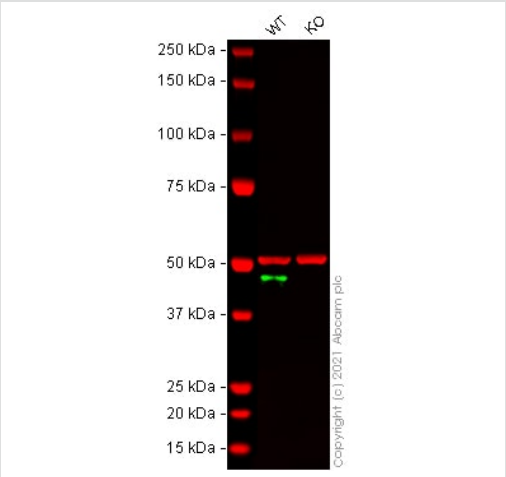
Application	Abreviews	Notes
WB		1/1000 - 1/10000. Predicted molecular weight: 39 kDa.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. Antigen retrieval is recommended.

Application notes Is unsuitable for Flow Cyt,ICC/IF or IP.

Target

Function	Scaffold protein that connects plasma membrane proteins with members of the ezrin/moesin/radixin family and thereby helps to link them to the actin cytoskeleton and to regulate their surface expression. Necessary for recycling of internalized ADRB2. Was first known to play a role in the regulation of the activity and subcellular location of SLC9A3. Necessary for cAMP-mediated phosphorylation and inhibition of SLC9A3. May enhance Wnt signaling. May participate in HTR4 targeting to microvilli (By similarity). Interacts with MCC.
Tissue specificity	Detected in liver, kidney, pancreas, prostate, spleen, small intestine and placenta, in particular in the syncytiotrophoblast.
Involvement in disease	Defects in SLC9A3R1 are the cause of hypophosphatemic nephrolithiasis/osteoporosis type 2 (NPHLOP2) [MIM:612287]. Hypophosphatemia results from idiopathic renal phosphate loss. It contributes to the pathogenesis of hypophosphatemic urolithiasis (formation of urinary calculi) as well to that of hypophosphatemic osteoporosis (bone demineralization).
Sequence similarities	Contains 2 PDZ (DHR) domains.
Post-translational modifications	Phosphorylated on serine residues.
Cellular localization	Cytoplasm. Apical cell membrane. Endomembrane system. Cell projection > filopodium. Cell projection > ruffle. Cell projection > microvillus. Translocates from the cytoplasm to the apical cell membrane in a PODXL-dependent manner (By similarity). Colocalizes with actin in microvilli-rich apical regions of the syncytiotrophoblast. Found in microvilli, ruffling membrane and filopodia of HeLa cells. Present in lipid rafts of T-cells.

Images



Western blot - Anti-EBP50/NHERF-1 antibody [EPR5562] (ab109430)

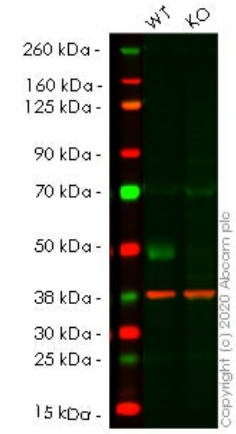
All lanes : Anti-EBP50/NHERF-1 antibody [EPR5562] (ab109430) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : SLC9A3R1 knockout HeLa cell lysate

Performed under reducing conditions.

Predicted band size: 39 kDa
Observed band size: 46 kDa

False colour image of Western blot: Anti-EBP50/NHERF-1 antibody [EPR5562] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab109430 was shown to bind specifically to EBP50/NHERF-1. A band was observed at 46 kDa in wild-type HeLa cell lysates with no signal observed at this size in SLC9A3R1 knockout cell line **ab264914** (knockout cell lysate **ab257280**). To generate this image, wild-type and SLC9A3R1 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged.Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-EBP50/NHERF-1 antibody [EPR5562] (ab109430)

All lanes : Anti-EBP50/NHERF-1 antibody [EPR5562] (ab109430) at 1/1000 dilution

Lane 1 : Wild-type HCT116 cell lysate
Lane 2 : SLC9A3R1 knockout HCT116 cell lysate

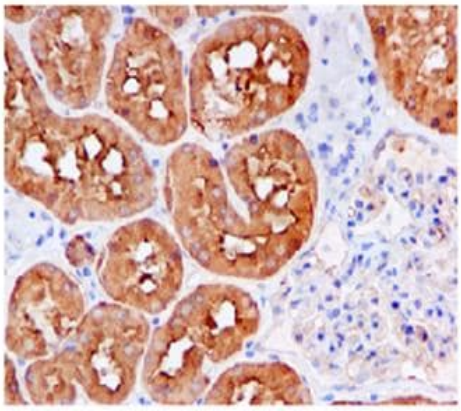
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 39 kDa
Observed band size: 48 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab109430 observed at 48 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

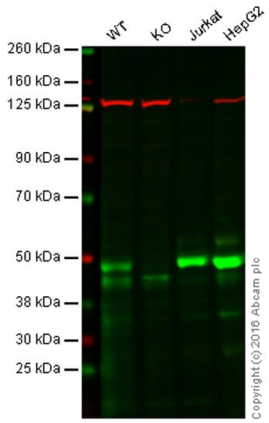
ab109430 was shown to react with EBP50/NHERF-1 in wild-type HCT116 cells in western blot. Loss of signal was observed when knockout cell line **ab266876** (knockout cell lysate **ab257281**) was used. Wild-type HCT116 and SLC9A3R1 knockout HCT116 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab109430 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EBP50/NHERF-1 antibody [EPR5562] (ab109430)

Immunohistochemical analysis of EBP50/NHERF-1 in paraffin-embedded Human kidney tissue using ab109430 at 1/100 dilution.

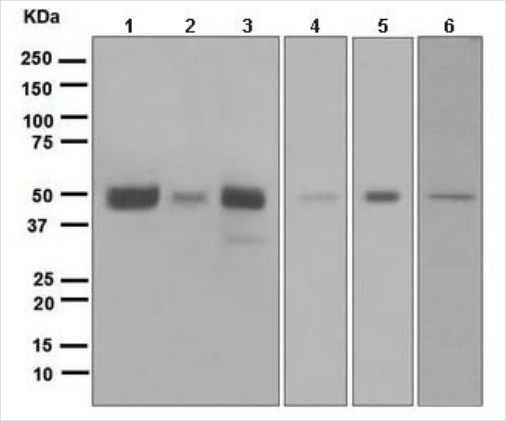
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Western blot - Anti-EBP50/NHERF-1 antibody [EPR5562] (ab109430)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: EBP50/NHERF-1 knockout HAP1 cell lysate (20 µg)
Lane 3: Jurkat cell lysate (20 µg)
Lane 4: HepG2 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab109430 observed at 48 kDa. Red - loading control, **ab18058**, observed at 124 kDa.

ab109430 was shown to specifically recognize EBP50/NHERF-1 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when EBP50/NHERF-1 knockout samples were usexamined. Wild-type and EBP50 knockout samples were subjected to SDS-PAGE. ab109430 and **ab18058** (loading control to Vinculin) were diluted at 1/500 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed withGoat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-EBP50/NHERF-1 antibody [EPR5562] (ab109430)

All lanes : Anti-EBP50/NHERF-1 antibody [EPR5562] (ab109430) at 1/1000 dilution

Lane 1 : HepG2 cell lysate
Lane 2 : 293T cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : C6 cell lysate
Lane 5 : PC12 cell lysate
Lane 6 : MCF7 cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 39 kDa

Anti-EBP50/NHERF-1 antibody [EPR5562] (ab109430)

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