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Datasheet for 600-401-A61

CEBP Delta Antibody

Overview

Description:	Anti-C/EBP delta (RABBIT) Antibody - 600-401-A61
Item No.:	600-401-A61
Size:	100 µg
Applications:	ELISA, WB, IHC, IP
Reactivity:	Mouse
Host Species:	Rabbit

Product Details

Background:	This antibody is designed, produced, and validated as part of a collaboration between Rockland and the National Cancer Institute (NCI) and is suitable for Cancer, Immunology and Nuclear Signaling research. C/EBP proteins are DNA-binding proteins that are important transcriptional activators in the regulation of genes involved in immune and inflammatory responses and may play an important role in the regulation of the several genes associated with activation and/or differentiation of macrophages. Members of the C/EBP transcription factor family are related by a high degree of amino acid sequence identity to the basic leucine zipper DNA-binding domain and show distinct but overlapping patterns of tissue- and stage-restricted expression. Osada et al. identified the consensus binding site for the family as RTTGCGYAAY (R = A or G, and Y = C or T). Phosphorylation of C/EBPd may increase binding activity, whereas phosphorylation of the a and b family members may decrease binding affinity. C/EBPd is a nuclear protein that binds DNA as a dimer and can form stable heterodimers with all other C/EBP family members.
Synonyms:	rabbit anti-CEBP Delta Antibody, C/EBP related protein 3 antibody, CCAAT/enhancer binding protein delta antibody, CEBP D antibody, CEBPD antibody, CELF antibody, Crp 3 antibody, Crp3 antibody, NF IL6 beta antibody, Nuclear factor NF IL6 beta antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	Cebpd

Reactivity:	Mouse
Immunogen Type:	Recombinant Protein
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a recombinant protein corresponding to the amino terminus of mouse C/EBP.
Purity/Specificity:	This product was affinity purified from monospecific antiserum by immunoaffinity chromatography. This antibody recognizes endogenous mouse and recombinant human C/EBP delta protein. A BLAST analysis was used to suggest cross-reactivity with C/EBPd from human sources based on 77% homology with the immunizing sequence. No cross-reactivity is observed with mouse C/EBPa or C/EBPb. Reactivity with C/EBPd from other sources has not been determined.
Relevant Links:	• UniProtKB - Q00322 • NCBI - 110347410 • GeneID - 12609

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	IHC, IP (Based on references)
Application Note:	This affinity purified antibody has been tested for use in ELISA, western blotting, and suitable in IHC. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 35 kDa in size corresponding to C/EBPd by western blotting in the appropriate cell lysate or extract. The predicted molecular weight of mouse C/EBPd is 28.8 kDa. This antibody is capable of detecting both over-expressed and endogenous C/EBPd.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:4,000 – 1:9,000
IHC:	1:2000
IP:	0.8 – 1.5 µg/ml
WB:	1:1,000 - 1:15,000

Formulation

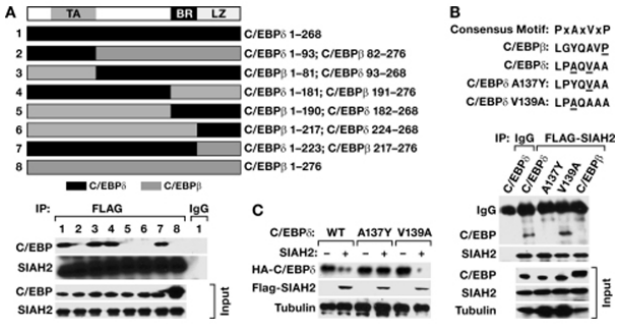
Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

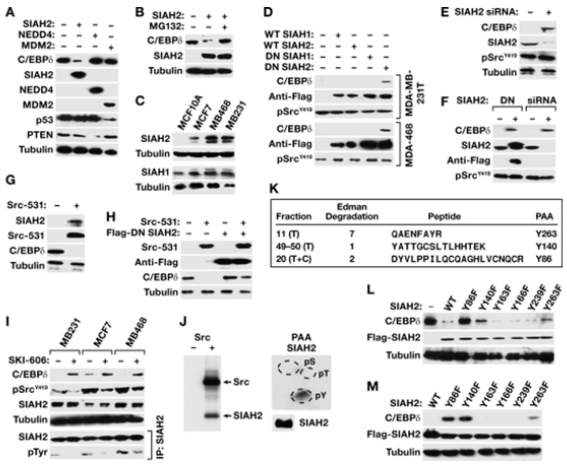
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

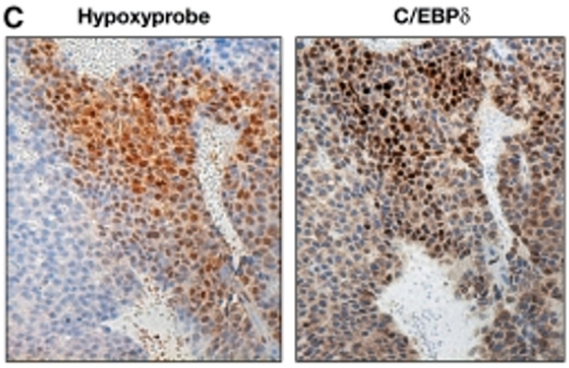


Western Blot
Identification of the SIAH2 interaction domain in C/EBPδ. (A) Six different C/EBPδ-C/EBPβ chimeric proteins, as shown in the schematic and the full-length proteins, were cotransfected with Flag-SIAH2 in MCF-10A cells. Immunoprecipitates with anti-Flag antibody and input were analyzed with a pan-C/EBP and anti-Flag antibody for SIAH2. Cells had been treated with MG132 to stabilize C/EBPδ. TA, transactivation domain; BR, basic region; LZ, leucine zipper. (B) Schematic of the sequence of human C/EBPδ and C/EBPβ aligned with the consensus for SIAH2 interaction domains and two point mutations in C/EBPδ (underlined amino acids match the consensus motif). Immunoprecipitates with anti-Flag antibody and input were analyzed with antibodies specific for C/EBPβ and C/EBPδ and with anti-Flag to detect SIAH2. Cells had been treated with MG132 to stabilize C/EBPδ. (C) Western analysis of MCF-10A cells after transfection with the indicated C/EBPδ expression constructs and Flag-SIAH2. Fig 5. PMID: 22037769

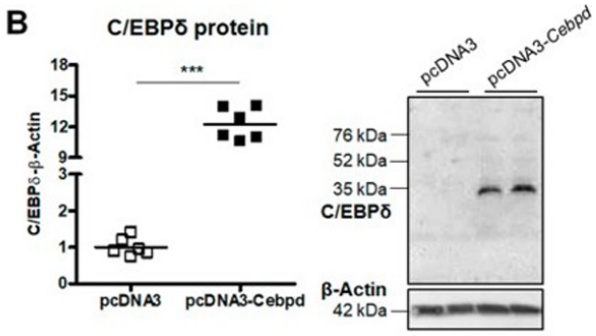


Western Blot

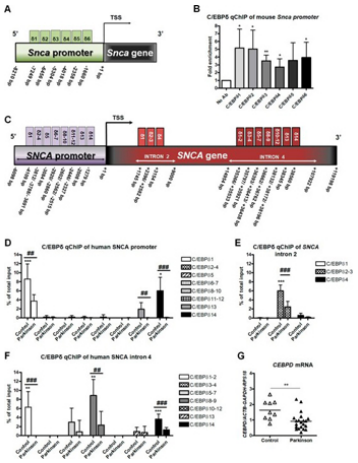
SIAH2 is necessary and sufficient for C/EBPδ downregulation. (A) Western analysis of MCF-10A cells transfected with expression constructs for the indicated proteins. (B) MCF-10A cells were transfected with SIAH2, treated with MG132, and analyzed as described in panel A. (C) Western analysis of the indicated cell lines for SIAH1 and SIAH2 expression. (D) Western analysis of MDA-MB-231T (top) and MDA-MB-468 (bottom) cells transfected with the indicated SIAH proteins, which were detected with anti-Flag antibodies. (E) Western analysis of MDA-MB-231T cells after nucleofection of siRNA against SIAH2. (F) Western analysis of MDA-MB-231T cells transfected with a DN-SIAH2 expression construct or nucleofected with siRNA against SIAH2. Anti-Flag was used to detect specifically DN-SIAH2, which has a higher MW than endogenous SIAH2. (G) Western analysis of MCF-10A cells transfected with Src-531. (H) Western analysis of MCF-10A cells cotransfected with Src-531 and/or DN-SIAH2. (I) Western analysis of the indicated cell lines treated with SKI-606 for 24 h. The same lysates were immunoprecipitated with anti-SIAH2 and immunoblotted with anti-phospho-tyrosine. (J) In vitro phosphorylation assay conducted by incubating affinity purified Flag-SIAH2 with (+) or without (−) purified Src-530 in the presence of [32P]ATP. Samples were resolved by SDS-PAGE, transferred to nitrocellulose, and subjected to autoradiography (left panel). Labeled SIAH2 was digested from the membrane and subjected to phosphoamino acid analysis (PAA; right panel). Expression of the input SIAH2 by Western analysis is also shown. (K) Results from HPLC analysis of SIAH2 with or without individual tyrosine-to-phenylalanine mutations (see panel L) after in vitro phosphorylation assays as in panel J, compared to unphosphorylated WT SIAH2, Edman degradation, and phosphoamino acid analysis. T, trypsin; T +C; trypsin followed by chymotrypsin. (L) Western analysis of MCF-10A cells transfected with expression constructs for the indicated SIAH2 proteins. (M) Western analysis of MDA-MB-231 cells transfected as described for panel L.
Fig 3. PMID: 22037769



Immunohistochemistry
(C) C/EBPδ is induced by hypoxia in vivo. Immunohistochemistry of parallel sections of an MMTV-Neu mammary tumour stained with anti-hypoxyprobe and anti-C/EBPδ antibodies. Original magnification: × 400. Fig 1. PMID: 21076392

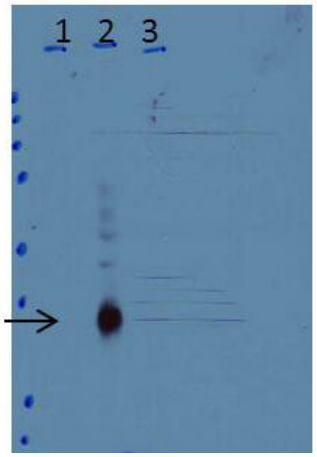


Western Blot
b) C/EBPδ protein increases 12.2-fold ($p < 0.0001$) in pcDNA3-Cebpd cerebellar granular cultures. C/EBPδ protein is assessed in cerebellar granular cultures by western blot using β-actin as the normalizing protein. **$p < 0.01$ and ***$p < 0.001$, using Student's t-test. Fig 2. PMID: 31209363

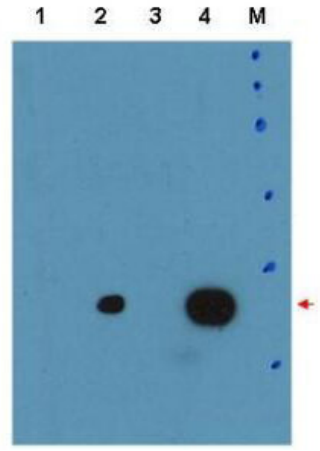


ChIP

C/EBPδ qChIP of mouse Snca and human SNCA genomic regulatory regions. a Schematic representation of the mouse Snca promoter showing the localization of six possible binding sites (named δ1, δ2, δ3, δ4, δ5 and δ6) for the transcription factor C/EBPδ in the Snca promoter region. b C/EBPδ protein binding to several Snca promoter boxes in granular neurons (seven independent experiments) using qChIP: δ1 ($p = 0.0223$), δ2 ($p = 0.0236$), δ3 ($p = 0.0045$), δ4 ($p = 0.0371$), and δ6 ($p = 0.0372$). c Schematic representation of the human SNCA genomic regions showing that the transcription factor C/EBPδ has fourteen possible binding sites in the SNCA promoter (named δ1, δ2–4, δ5, δ6–7, δ8–10, δ11–12, and δ14), four possible binding sites in the SNCA intron 2 (named δ1, δ2–3, and δ4), and fourteen possible binding sites in the SNCA intron 4 (named δ1–2, δ3–4, δ5–7, δ8–9, δ10–12, δ13, and δ14). d C/EBPδ protein binding to two SNCA promoter boxes in human cerebral cortex using qChIP: δ1 ($p < 0.0001$) and δ14 ($p < 0.05$). A significant decrease in C/EBPδ binding to human SNCA promoter boxes δ1 ($p = 0.0018$), δ13 ($p = 0.0026$), and δ14 ($p < 0.0001$), by qChIP was observed in cerebral cortex samples from PD patients ($n = 8$) vs non-neurological controls ($n = 8$). e C/EBPδ protein binding to SNCA intron 2 boxes in human cerebral cortex using qChIP: δ2–3 ($p < 0.0001$). A significant decrease in C/EBPδ binding to human SNCA intron 2, boxes δ2–3 ($p = 0.0004$) by qChIP is observed in cerebral cortex samples from PD patients ($n = 7$) vs non-neurological controls ($n = 6$). f C/EBPδ protein binding to SNCA intron 4 boxes in human cerebral cortex using qChIP: δ1–2 ($p < 0.01$), δ8–9 ($p < 0.01$), and δ14 ($p < 0.001$). A significant decrease in C/EBPδ binding to human SNCA intron 4 boxes δ1–2 ($p = 0.0001$), δ8–9 ($p = 0.0015$), and δ14 ($p < 0.0001$) by qChIP is observed in cerebral cortex samples from PD patients ($n = 8$) vs non-neurological controls ($n = 8$). g Expression of CEBPD mRNA in substantia nigra samples from non-neurological controls ($n = 9$) and PD ($n = 21$) patients. A significant downregulation in CEBPD mRNA ($p = 0.0037$) is observed in PD samples when compared with control samples. Bars show mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs respective no Ab, and ##$p < 0.01$, ###$p < 0.001$ vs respective control, using Student's t-test. Fig 4. PMID: 31209363



Western Blot
Western Blot of Anti-C/EBPd Antibody. Lane 1: untreated wild-type (WT) cells. Lane 2: WT cells treated 3 h with LPS. Lane 3: C/EBPd-knock-out cells treated 3H with LPS. Load: 35 µg per lane. Primary: Anti-C/EBPd Antibody used at a dilution of 1:15k overnight at 4°C. Blocking: buffer TBST. ECL was used for detection of endogenous protein in whole cell extracts from mouse primary macrophage cells. Predicted/Observed size: 28.8 kDa, ~35 kDa.



Western Blot
Western Blot of Anti-C/EBPd Antibody Lane 1: human C/EBPbeta Lane 2: Mouse C/EBPdelta Lane 3: untransfected Lane 4: human C/EBPdelta Load: 35 µg per lane Primary: Anti-C/EBPd Antibody used at a dilution of 1:15k overnight at 4°C Blocking: buffer TBST ECL was used for detection of overexpressed protein in human and mouse C/EBP delta. Predicted/Observed size: 28.8 kDa, ~35 kDa

References

- Valente T et al. CCAAT/enhancer binding protein δ is a transcriptional repressor of α-synuclein. *Cell Death Differ.* (2020)
- Sarkar TR et al. Identification of a Src tyrosine kinase/SIAH2 E3 ubiquitin ligase pathway that regulates C/EBPδ expression and contributes to transformation of breast tumor cells. *Mol Cell Biol.* (2012)
- Balamurugan K et al. The tumour suppressor C/EBPδ inhibits FBXW7 expression and promotes mammary tumour metastasis. *EMBO J.* (2010)

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