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Datasheet for 100-401-225

Dab1 Antibody

Overview

Description:	Anti-Dab1 (400-555) (RABBIT) Antibody - 100-401-225
Item No.:	100-401-225
Size:	100 µL
Applications:	IHC, WB, Biochemical Assay, IF, Multiplex
Reactivity:	Mouse
Host Species:	Rabbit

Product Details

Background:	Anti-Dab1 Antibody recognizes Dab1 that is a phosphoprotein encoded by the mouse gene dab and is related to the Drosophila gene 'disabled'. Mutations in the mouse dab gene may result in the 'scrambler' and 'yotari' phenotypes. Dab1 binds to non-receptor tyrosine kinases and plays an important role in brain development. Dab1 is expressed in neuronal populations exposed to reelin, and it functions as a signaling molecule that regulates cell positioning in the developing brain. Cloning of human Dab1 and sequence determinations suggest a 96% identity to the mouse sequence. Dab1 binds to cytoplasmic regions of very low density lipoprotein receptors (VLDLR), apolipoprotein E receptor-2 (ApoER2) and the Amyloid Precursor Protein (APP) family of proteins. Dab1 accumulates in ectopic neurons from mice lacking Reelin or both VLDLR and ApoER2. In humans, Dab1 has been mapped to lp32-p312. This region shows homology of synteny with the segment of mouse chromosome 4 containing Dab1.
Synonyms:	rabbit anti-Dab 1 antibody, Disabled homolog 1 antibody, Disabled homolog 1 Drosophila antibody, Scm antibody, Scr antibody, Scrambler antibody, Yot antibody, Yotari antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	Antiserum

Target Details

Gene Name:	Dab1
Reactivity:	Mouse

Immunogen Type:	Conjugated Peptide
Immunogen:	This whole rabbit serum was prepared by repeated immunizations with a synthetic peptide corresponding to the C-terminal region of murine Dab1 at amino acids 400-555.
Purity/Specificity:	Dab-1 whole rabbit antiserum was prepared by delipidation and defibrination followed by the addition of buffer salts and preservative. This antibody is directed against Dab1 from mouse. Cross-reactivity with other species has not been determined. No reaction occurs with human or mouse Dab2.
Relevant Links:	• UniProtKB - P97318 • NCBI - NP_034144.1 • GeneID - 13131

Application Details

Tested Applications:	IHC, WB
Suggested Applications:	Biochemical Assay, IF, Multiplex (Based on references)
Application Note:	Anti-Dab1 antibody has been tested by western blot and immunohistochemistry and is suitable for the detection of Dab1 by immunoprecipitation. Specific conditions for reactivity should be optimized by the end user. Expect a band at 80 kDa corresponding to Dab1 in the appropriate tissue extract or cell lysate. For western blotting block the blot using 5% BLOTTO for 1 h at room temperature and followed by incubation with the primary antibody diluted in 1% BLOTTO in TTBS for 1 h at room temperature. For immunoprecipitation use 1 µl of antiserum per 500 µg of brain lysate. Perform immunoprecipitation at 4°C for 2 h. For immunoprecipitation buffer lysates with 50 mM Tris-Cl, pH 7.4, supplemented with 150 mM sodium chloride, 1% (v/v) NP-40, 10 µg/ml aprotinin and 10 µg/ml leupeptin.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:50,000
IF:	User Optimized
IHC:	1:5,000
IP:	1 µl per 500 µg
WB:	1:5,000

Formulation

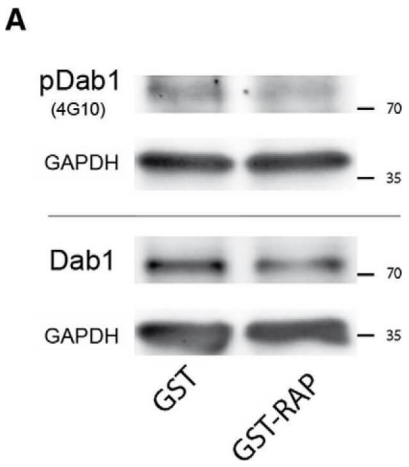
Physical State:	Liquid (sterile filtered)
Concentration:	75 mg/mL by Refractometry

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

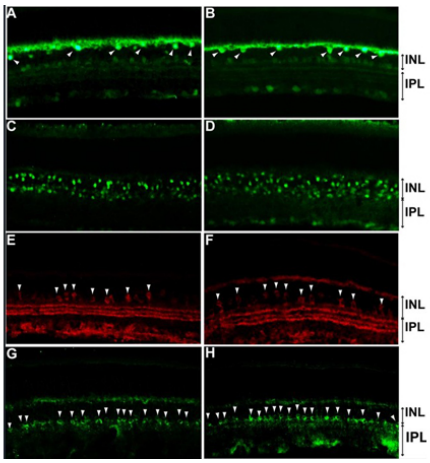
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several 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

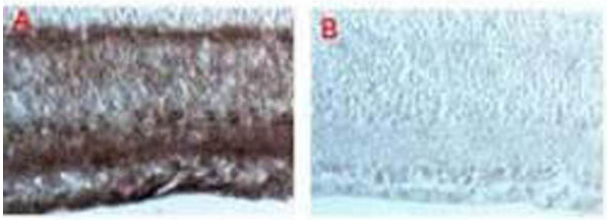


Western Blot

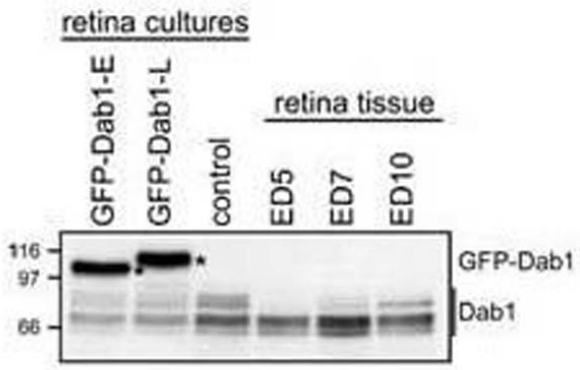
Coapplication of GST-RAP does not prevent the E2-induced distal dendritic HCN1 enrichment in vitro. A, Western blots showing phosphorylated Dab1 (pDab1, upper bands, detected by anti-phosphotyrosine antibody 4G10) and total Dab1 (lower bands) in “paired” slice cultures that were treated for 24 h (DIV10–11) with either GST-RAP or GST (10 µg/ml each) and were exposed to Reelin-conditioned medium for 30 min before harvesting. GAPDH was used for loading control. B, Quantitative analysis of Dab1 and pDab1 levels (relative to GAPDH) revealed that Reelin-induced pDab1 was reduced in the GST-RAP-treated slices, while total Dab1 was not significantly different compared to the GST-treated controls. Thus, pDab1/Dab1 was reduced to 72% ± 6% of control levels after 24-h GST-RAP treatment ($p < 0.01$; $n = 15$). C, D, E2 (+GST)-treatment (pink) of cultures for 6 d (DIV5–11) caused a significant HCN1 accumulation in segment 5 (C) and a significantly increased slope (D) compared with controls (GST, black) that was not efficiently reduced, if GST-RAP (10 µg/ml) was coapplied (orange; $n = 17$, each group). E–G, Representative photographs from a culture “triple,” of which one culture served as a vehicle (GST)-treated control (E), while the others were treated with either E2 + GST (F) or E2 + GST-RAP (G). Note that HCN1 is accumulated at the hippocampal fissure (asterisks) at all conditions, but if E2 was present (F, G), less HCN1 immunosignal is visible in stratum radiatum (sr, arrows), indicating (relative) HCN1 enrichment in stratum lacunosum-moleculare (slm). Scale bars: 80 µm (E–G). Dashed lines demarcate the border of stratum pyramidale (sp). ml, molecular layer. Figure provided by CiteAb. Source: Eneuro, PMID: 30406178.



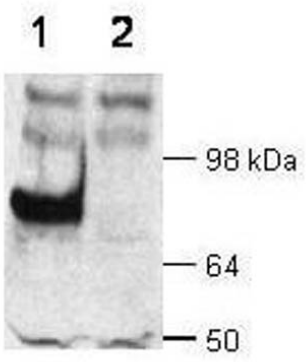
Immunofluorescence Microscopy
Immunofluorescent labeling of INL cell types in adult wild-type (A, C, E, and G) and Bhlhb4 knockout (B, D, F, and H) retinas.
(A and B) Horizontal cells (arrows) labeled by anti-calbindin D antibodies. (C and D) Anti-caldendrin antibodies label an OFF cone bipolar cell subset. (E and F) Cholinergic (starburst) amacrine cell somata and processes and displaced amacrine cells labeled by anti-calretinin. (G and H) AII amacrine cells were labeled by anti-DAB1 at 1:1000. All four cell-types shown remained constant in number and morphology in the Bhlhb4 knockout as compared to wild-type. The inner nuclear layers (INL) and the inner plexiform layers (IPL) are indicated. Supplemental Figure S1 PMID: 15363390.



Immunohistochemistry
Immunohistochemical staining after cryofixation and sectioning of mouse brain tissue using (A) a 1:5,000 dilution of anti-Dab-1 and (B) 1:5,000 dilution of pre-immune serum followed by processing with HRP Goat anti-Rabbit IgG [H&L] and chromogenic substrate.



Western Blot
Analysis of GFP-Dab1 and endogenous Dab1 levels in transfected retinal cells and retinal tissue. Western blot analysis of whole cell lysates prepared from primary retinal cultures transfected with GFP-Dab1-E (lane 1), -L (lane 2), control untransfected retinal cultures (lane 3), and retinal tissue at ED5 (lane 4), ED7 (lane 5) and ED10 (lane 6). Proteins were electrophoresed through an SDS-8% polyacrylamide gel and transferred to nitrocellulose. The membrane was immuno-stained with Rockland Immunochemical's anti-Dab1 antibody at 1:5000, which recognizes both GFP-Dab1 (indicated by asterisks) and endogenous forms of Dab1 (indicated by a line). See Katval et al (2007) for additional details.



Western Blot
Rockland Immunochemical's anti-Dab1 is shown to detect Dab1 present in wt mouse brain extracts (lane 1). No staining is noted in similar extracts from a dab knock-out mouse (lane 2). Detection of an 80 kDa band (arrowhead) occurs using a 1:5,000 dilution of the antibody in 1% milk in TTBS for 1 h at room temperature followed by a 1:5,000 dilution of HRP Goat-a-Rabbit with ECL visualization. Film exposure was ~1'. Other detection systems will yield similar results.

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