Datasheet for ABIN6254097

anti-CALB1 antibody

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Overview

Quantity:	100 µL
Target:	CALB1
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This CALB1 antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (IHC), Immunofluorescence (IF)

Product Details

Immunogen:	Recombinant full length human calbindin purified from E. coli.
Isotype:	IgG2a
Cross-Reactivity:	Cow (Bovine), Human, Mouse (Murine), Rat (Rattus)
Purification:	Protein G purified culture supernatant

Target Details

Target:	CALB1
Alternative Name:	CALB1 (CALB1 Products)
Molecular Weight:	'28 kDa
Gene ID:	793
UniProt:	P05937

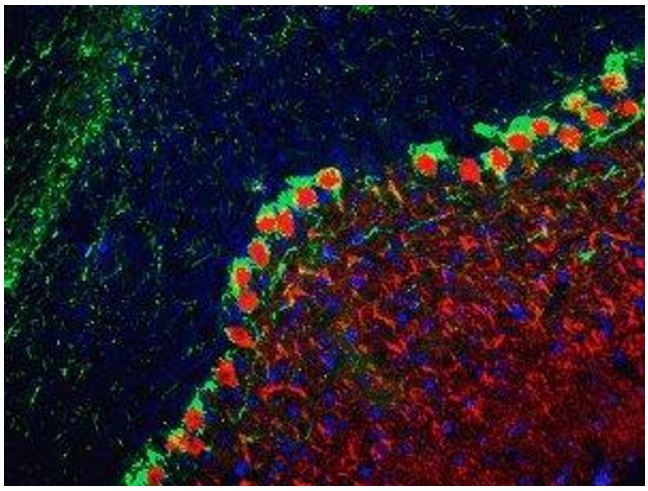
Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Restrictions:	For Research Use only

Handling

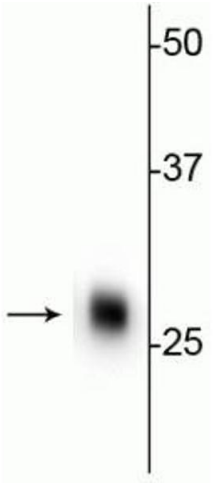
Buffer:	100 µL in PBS + 50 % glycerol and 5 mM Sodium azide
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C

Images



Immunostaining

Image 1. Immunostaining of rat cerebellum showing specific labeling of calbindin (red) in the dendrites of Purkinje cells. Axons are stained green with anti-neurofilament H antibody (ABIN361351).



Western Blotting

Image 2. Western blot of rat cerebellar lysate showing specific immunolabeling of the ~ 28 kDa calbindin protein.

Successfully validated (Immunohistochemistry (IHC))

by Prof. Merighi, Laboratory of Neurobiology, Department of Veterinary Sciences, University of Turin

Report Number: 104494

Date: Aug 02 2023

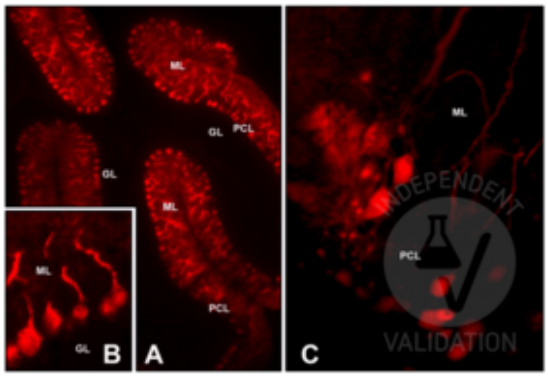
Target:	CALB1
Lot Number:	GS116G
Method validated:	Immunohistochemistry (IHC)
Positive Control:	Adult (>2 months) CD1 mouse cerebellum (6 µm glass-mounted microtome sections) Postnatal day 6-7 CD1 mouse cerebellum (cultured cerebellar slices)
Negative Control:	Control slices were processed for each experimental procedure, omitting the primary antibody; overnight incubation in diluent solution only.
Notes:	Passed. The CALB1 antibody ABIN6254097 works in IHC-P at a 1:50 dilution with or without tyramide amplification.
Primary Antibody:	ABIN6254097
Secondary Antibody:	goat anti-mouse IgG (H+L) AF488-conjugated antibody (Thermo Fisher Scientific, A11034, lot 2380031)
Protocol:	• Indirect IMF on microtome sections ◦ Perfuse adult (>2 months) CD1 mice with paraformaldehyde 4% in 0.1 M phosphate buffer pH 7.4 and post-fix in the same fixative for an additional 2 h at RT. ◦ Wash, dehydrate, and embed samples in paraffin wax. ◦ Wash several times with 0.01 M PBS. ◦ Cut the cerebellum with a microtome into 6 µm sections and mount them on glass slides. ◦ After paraffin removal, incubate sections for 1 h at RT in PBS containing 1% albumin from chicken egg white (Sigma, A5378) and 0.3% Triton-X-100 (BioRad, 161-0407, lot 00583) to block non-specific binding sites. ◦ Incubate sections with primary mouse anti-CALB1 antibody (antibodies online, ABIN6254097, lot GS116G) diluted 1:50, 1:100, and 1:200 in 0.1 M PBS-BSA-PLL ON at RT. ◦ Wash 3x 5 min in 0.01 M PBS. ◦ Incubate sections with secondary goat anti-mouse IgG (H+L) AF488-conjugated antibody (Fisher Scientific, A11034, lot 2380031) diluted 1:500 in 0.1 M PBS for 1 h at RT. ◦ or alternatively with tyramide amplification: ◦ Incubate sections with Poly-HRP-conjugated secondary antibody for 1 h at RT. ◦ Wash sections 3x 5 min in 0.01 M PBS.

- Incubate sections with Tyramide working solution (for 5 sections: 5 µL 100X Tyramide stock solution, 5 µL 100X H_2O_2 solution, 500 µL 1X Reaction buffer) for 10 min at RT.
- Stop the reaction with the Reaction Stop Reagent working solution.
- Wash 3x 5 min in 0.01M PBS.
- Mount specimens in Fluoroshield (Sigma-Aldrich, F6182, lot MKCB0153V).
- Acquire Images with Leica DM 6000B fluorescence microscope equipped with a digital camera at 20-40x magnification.

- Indirect IMF on cultured cerebellar slices
  - Euthanize CD1 mice at postnatal day 6-7 (P6-P7) with an overdose of 60 mg⁄100 g body weight sodium pentobarbital (Merck Life Science, Y0002194).
  - Remove the brain removed from the skull while the head is kept submerged in ice-cooled Gey's solution (Sigma-Aldrich) supplemented with glucose and antioxidants (for 500 mL: 4.8 mL 50% glucose, 0.05 g ascorbic acid, 0.1 g sodium pyruvate).
  - Dissect the cerebellum from the brain.
  - Cut 350 µm thick parasagittal slices of the cerebellum with a McIlwain tissue chopper (Brinkmann Instruments).
  - Plate two to three slices onto a Millicell Cell Culture Insert (Merck Life Science, PICM0RG50).
  - Place each insert inside a 35 mm Petri dish containing 1 mL of culture medium consisting of 50 % Eagle basal medium (BME, Sigma Chemicals), 25 % horse serum (Gibco by Thermo Fisher Scientific), 25 % Hanks balanced salt solution (Sigma-Aldrich), 0.5 % glucose, 0.5 % 200 mM L-glutamine, and 1 % antibiotic/antimycotic solution.
  - Incubate slices at 34 °C in 5 % CO_2 for up to 20 d in vitro (DIV). Change the medium twice a week. Slices were allowed to equilibrate to the in vitro conditions for at least 4-6 DIV before IMF.
  - Remove the culture medium from the dish and fix the slices in 1 mL of 4 % paraformaldehyde (Merck Life Science, P6148) in PBS for 1 h.
  - Wash 3x 5 min in 0.01 M PBS.
  - Incubate fixed cultures in PBS containing 1 % Triton X-100 (BioRad, 161-0407, lot 00583) for 10 min.
  - Wash 3x 5 min in 0.01 M PBS.
  - Incubate cultures ON at 4 °C under continuous stirring in PBS containing 1 % albumin from chicken egg white (Sigma, A5378) and 0.3 % Triton-X-100 (BioRad, 161-0407, lot 00583) to block non-specific binding sites.
  - Incubate cultures with the primary mouse anti-CALB1 antibody (antibodies online, ABIN6254097, lot GS116G) diluted 1:50 in PBS-BSA (Sigma, A7906)-PLL (Sigma, P1524) ON at RT.
  - Wash 5 x 5min in PBS.
  - Incubate cultures with the secondary anti-rabbit antibody Alexa Fluor 488 diluted (Invitrogen by Thermo Fisher Scientific, A11034, lot 2380031) 1:500 in 0.1 M PBS for 1 h at RT.
  - Wash 3x 5 min in 0.01 M PBS.
  - Mount specimens in Fluoroshield (Sigma-Aldrich, F6182, lot MKCB0153V).
  - Acquire Images with Leica DM 6000B fluorescence microscope equipped with a digital

	camera at 20-40x magnification.
Experimental Notes:	For indirect IMF on cerebellum paraffin sections, antigen retrieval treatment was also tested. In this case, sections were processed for microwave antigen retrieval for 10 min (95-100 °C) in 10 mM sodium citrate buffer (pH 6.0). After 20 min of spontaneous cooling, sections were washed twice for 5 min with distilled water and twice for 5 min with PBS.

Image for Validation report #104494



Validation image no. 1 for anti-Calbindin (CALB1) antibody (ABIN6254097)

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Validation report #104530 for Western Blotting (WB)

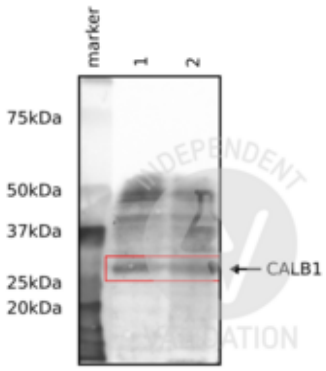
Successfully validated (Western Blotting (WB))

by Prof. Merighi, Laboratory of Neurobiology, Department of Veterinary Sciences, University of Turin

Report Number: 104530

Date: Aug 02 2023

Target:	CALB1
Lot Number:	GS116G
Method validated:	Western Blotting (WB)
Positive Control:	Adult mouse brain and cerebellum
Notes:	Passed. The CALB1 antibody ABIN6254097 works in WB at a 1:1000 dilution.
Primary Antibody:	ABIN6254097
Secondary Antibody:	goat anti-mouse IgG (H+L) HRP-conjugated (Thermo Fisher Scientific, G-21040)
Protocol:	• Homogenize tissues with cold lysis buffer containing 50 mM Tris HCl, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, and 1% protease inhibitor (Sigma P8340) using an ultrasonic homogenizer (MSE, SoniPrep 150) with 16 amplitude, 20 s on, 10 s off pulse for 60 s. • Centrifuge tissue homogenates at 13,000 rpm for 20 min at 4 °C. • Collect supernatants and determine total protein content using a Bradford assay. • Denature 100 µg of total protein for 5 min at 90 °C and subsequently separate them on a denaturing 12% PAGE-SDS gel alongside a Precision Plus Protein Dual Color Standard (Bio-Rad, 160374). • Electro-transfer proteins onto nitrocellulose membrane (Amersham Biosciences, RPN203D) ON in the cold room. • Wash membrane 3x for 10 min with 0.01 M PBS containing 0.1% Tween-20 (PBST). • Block membrane with PBST containing 2% bovine serum albumin for 1 h at RT. • Incubate membrane with primary rabbit anti-CALB1 antibody (antibodies-online, ABIN6254097, lot GS116G) diluted 1:1,000 in PBST ON at 4 °C. • Wash membrane 3x 10 min with PBST. • Incubate membrane with secondary HRP-conjugated goat anti-mouse IgG (Thermo Fisher Scientific, G-21040) diluted 1:50,000 in PBST for 1 h at RT. • Wash membrane 3x 10 min with PBST. • Visualize proteins with SuperSignal West Atto Ultimate Sensitivity Substrate (Thermo Fisher Scientific, A38555) using a ChemiDoc Imaging System.



Validation image no. 1 for anti-Calbindin (CALB1) antibody (ABIN6254097)

Western blot results using ABIN6254097 to reveal CALB1 (28 kDa) in the adult mouse brain and cerebellum.