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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Anti-Reelin (CR-50) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D223-3	RE-3B9 (R3B9)	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Reelin is a large extracellular glycoprotein of 420-450 kDa that controls cortical development and secreted by several neurons, such as cortical Cajal-Retzius cells. Defective Reelin is the cause of the *reeler* malformation in mouse and the Norman-Roberts type lissencephaly in human. Reelin is thought to deliver a signal to migrating neurons, instructing them to assume their correct position. Their response requires binding of Reelin to at least one of two lipoprotein receptors, very-low-density lipoprotein receptor (VLDLR) and apolipoprotein-E receptor type 2 (ApoER2), thereby inducing phosphorylation of the Dab1 (Disabled 1) adapter that interacts with the cytoplasmic tail of receptors. Anti-Reelin monoclonal antibody CR-50 interferes with Reelin homopolymerization and with Dab1 phosphorylation. Intraventricular injection of CR-50 disrupts the organized development of the hippocampus, resulting in a pattern similar to that found in *reeler*.

SOURCE: This antibody CR-50 was purified from hybridoma (clone RE-3B9) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with *reeler* mutant mice splenocyte immunized with homogenates of normal embryonic brain.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

INTENDED USE:
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REACTIVITY: This antibody reacts with mouse Reelin. The CR-50 epitope is located between mouse Reelin amino acid 230 to 346[8].

APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; Not tested*
Immunohistochemistry;
Frozen-section; 2-10 µg/mL
Paraffin embedded section; Not recommended
Immunocytochemistry; Not tested*
Flow cytometry; Not tested

*It is reported that CR-50 could be used in Immunoprecipitation and Immunocytochemistry [8), 13)]. It is also reported that CR-50 interferes with the aggregation of Reelin, and blocks its function *in vitro* and *in vivo* [1), 2), 8), 9), 11), 12), 14), 15)].

Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Tissue	Not tested	Fetal brain	Not tested
Reactivity on IHC		+	

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7) Tabata, H. and Nakajima, K. *Neuroscience* **103**, 865-872 (2001)
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12) Miyata, T., *et al*., *J. Neurosci.* **17**, 3599-3609 (1997)
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Antibody CR-50 is used in these references.

PROTOCOLS:

Immunohistochemical staining for frozen sections

Fixation and Frozen-section

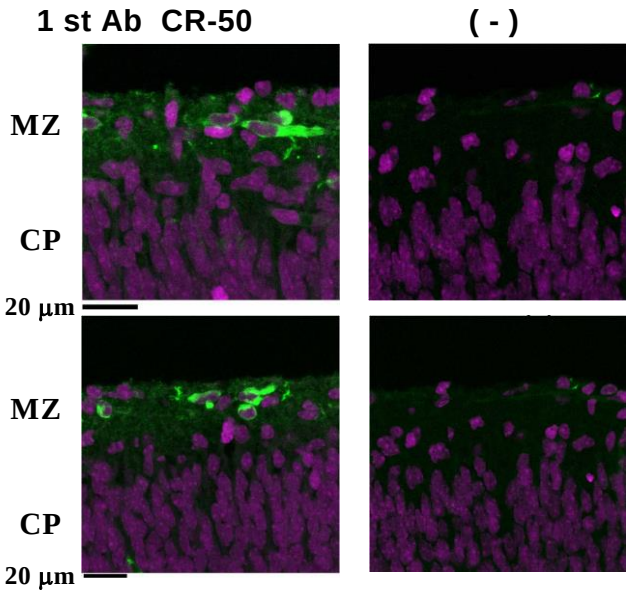
All animals were anesthetized on ice or with sodium pentobarbitone at concentration of 50 mg per gram of body weight.

1) Fix by perfusion of 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4).
2) The brain is dissected out and post fixed in 4% paraformaldehyde at 4°C.
3) Wash subject in phosphate-buffered saline (PBS) for 1 hour.
4) Equilibrate the samples in 20% sucrose in PBS.
5) Equilibrate the samples in 30% sucrose in PBS.
6) Embed brains in Tissue-Tek® O.C.T. Compound (Sakura; code no. 4583).
7) Freeze the brain samples with liquid nitrogen.
8) The frozen sections are cut coronally at 20 µm with a cryostat and mounted onto silane-coated glass slides.
9) Air dry with a fan for more than 1 hour at room temperature.

Immunohistochemistry

1) Remove the O.C.T. Compound by washing the samples in PBSTx [0.01% Triton X-100 in PBS] (10 minutes x 3 times).
2) Remove the slides from PBSTx, and after gently wiping off extra solution around each tissue section, immerse the tissues with blocking buffer (10% normal goat serum in PBSTx) for 1 hour at room temperature to block non-specific antibodies.
3) Pour out the blocking buffer, gently wipe around each section, and immerse the tissues with 1:100-1:500 anti-Reelin monoclonal antibody (CR-50) diluted with 5% normal goat serum in PBSTx. Incubate sections at 4°C overnight.
4) Wash the slides in PBSTx (10 minutes x 3 times).
5) Wipe gently around each section and immerse tissues with FITC-conjugated goat anti-mouse IgG.
6) Incubate for 1 hour at room temperature.
7) Wash the slides in PBSTx (10 minutes x 3 times).
8) Immerse the sections in PermaFluor™ aqueous mounting medium.
9) Observe the sections under a fluorescence microscope.

(Positive control for Immunohistochemistry; Mouse fetal brain)



Immunohistochemical detection of Reelin on frozen sections of mouse fetal brain (E18) with D223-3 (left) or isotypic control IgG (right). This data was kindly provided by Professor Kazunori Nakajima and Dr. Ken-ichiro Kubo (Department of Anatomy, Keio University School of Medicine, Tokyo).

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