**MONOCLONAL ANTIBODY**

**Anti-Reelin (CR-50) mAb**

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Clone</th>
<th>Subclass</th>
<th>Quantity</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D223-3</td>
<td>RE-3B9 (R3B9)</td>
<td>Mouse IgG1</td>
<td>100 µL</td>
<td>1 mg/mL</td>
</tr>
</tbody>
</table>

**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 is embryopathy 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:** For Research Use Only. Not for use in diagnostic procedures.

**REACTIVITY:** This antibody reacts with mouse Reelin. The CR-50 epitope is located between mouse Reelin amino acid 230 to 346.

**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 vivo13, 21, 8), 9), 11), 12), 14), 15).

Detailed procedure is provided in the following PROTOCOLS.

**SPECIES CROSS REACTIVITY:**

<table>
<thead>
<tr>
<th>Species</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactivity on IHC</td>
<td>Not tested</td>
<td>Fetal brain</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

**REFERENCES:**
7) Tabata, H. and Nakajima, K. *Neuroscience* 103, 865-872 (2001)

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.

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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)

**RELATED PRODUCTS:**
- M067-3 Anti-Apolipoprotein E4 (Human) mAb (1F9)
- M068-3 Anti-Apolipoprotein E (Human) mAb (3D12)
- D273-3 Anti-ApoER2 (LA8) (Mouse) mAb (25G5)
- 7635 ApoE4/Pan-ApoE ELISA Kit

*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).*