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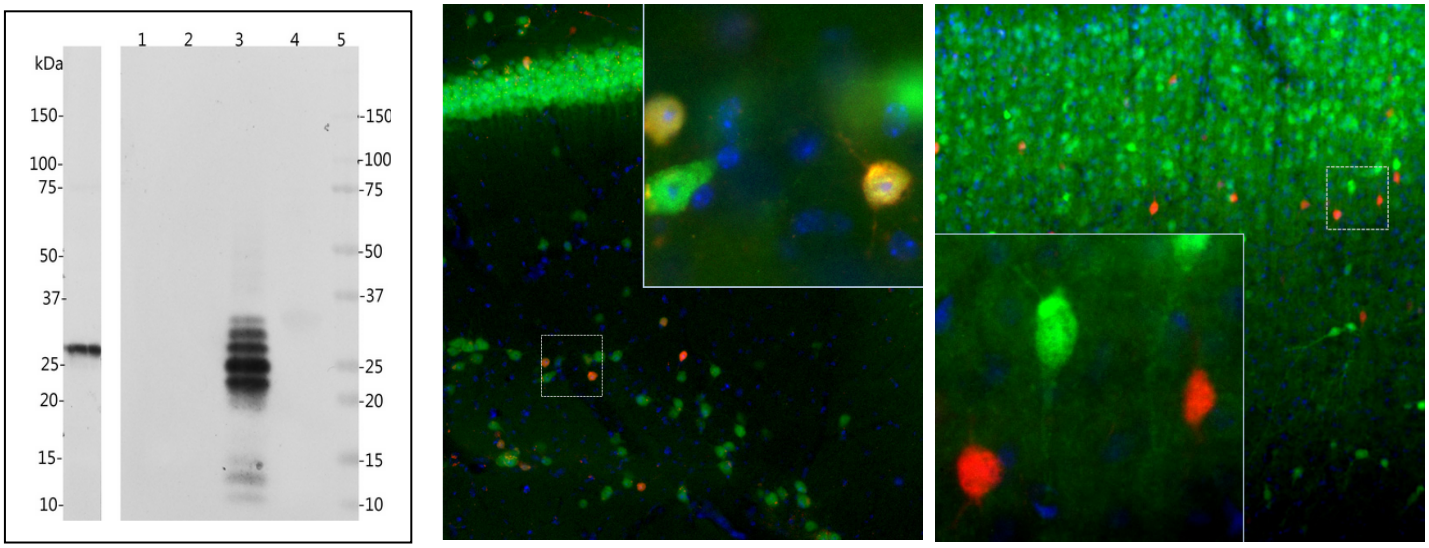
Catalogue# MCA-3G9: Mouse Monoclonal Antibody to Calretinin

The Immunogen: Calretinin, first described in 1987, acquired its name based on homology with calcium binding protein, calbindin D28k and the tissure of first detection (Chick retina) (1). As a member of the large superfamily of cytoplasmic Ca^{2+} binding proteins, Calretinin belongs to the subclass of these containing the "EF hand" Ca^{2+} binding motif originally characterized in parvalbumin (2). Calretinin is expressed in mammalian central nerve system, testis, fallopian tube and pancreas. In the brain it is localized in certain classes of neurons, and antibodies to it are useful for identifying specific neuronal cell types (3). It is particularly concentrated in granular cells and their parallel fibres, but is also found in many GABAergic interneurons in the cortex. These GABAergic interneurons in most cases express only one of three Ca2+ binding proteins, namely calretinin, calbindin or parvalbumin. As a result these important inhibitory interneurons can be identified and subclassified based on their content of these three proteins (3). Each type of neuron as defined in this fashion has particular electrophysiological and functional properties. For example, calbindin positive interneurons are not fast-spiking as are parvalbumin expressing interneurons. Human calretinin is also known as 29 kDa calbindin and calbindin-2, for its sequence is related to calbindin. Calretinin contains six EF-hand domains. Four of them bind Ca2+ with high affinity in a cooperative manner, one with low affinity and the last one is non-functional, without Ca2+-binding ability (4,5). The function of calretinin appears to be primarily buffering the Ca^{2+} level in cells and affect intracellular calcium signals. Calretinin deficiency in mossy cells of dentate gyrus and granule cells results abnormal excitability in the cerebellar neuronal network and impairment of long-term potentiation and motor coordination (6). The HGNC name for this protein is CALB2.

MCA-3G9 was raised against human calretinin protein expressed in and purified from *E.coli*. This antibody does not cross-react with the related calcium binding proteins 28kD calbindin, parvalbumin and secretigogin.



Left: Western blot analysis of MCA-3G9. Blot of cow cerebellum lysate (left blot) was probed with MCA-3G9. The MCA-3G9 binds strongly and cleanly to the calretinin band at 29kDa. Right: Blot of recombinant protein: secretagogin (lane 1), parvalbumin (lane 2), calretinin (lane 3), calbindin (lane 4) was probed with MCA-3G9. Lane 5 is protein marker. This antibody recognizes only calretinin, not other calcium-binding proteins. **Middle:** Adult mouse brain hippocampal section (45 µM; fixed by transcardial perfusion with 4% paraformaldehyde) was co-stained with MCA-3G9 (1:1000, red) and our rabbit polyclonal antibody to Fox3/NeuN (**RPCA-Fox3**, green). Calretinin stains a small number of interneuron in the stratum radiatum of CA1 region, and Fox/NeuN is expressed in most neurons in the brain. As a result, cells are positive for calretinin appear to be yellow. **Right**: Adult rat cortical section (45 µM; fixed by transcardial perfusion with 4% paraformaldehyde) was co-stained with MCA-3G9 (red) and our chicken polyclonal antibody to calbinidin (**CPCA-Calbindin**; green). In cortex, calretinin is expressed in a small population of interneurons concentrated in Layer 4 area, while calbindin is expressed in cells concentrated in Layer 2/3. Because each antibody specifically labels a different population of cells exclusively, the cells are either stained with green, or red in the cortex. Insets are high-magnification images of the boxed area in each picture. Blue is Dapi staining that labels DNA.

Antibody Characteristics: MCA-3G9 is a mouse IgG1 class antibody. The antibody was raised against full-length recombinant human calretinin protein expressed in and purified from *E.coli*. The antibody solution is purified from tissue culture supernatant at a concentratin of 1 mg/mL in phosphate buffered saline. This antibody is specific to calretinin, and does not cross-react with the related calcium binding proteins 28kD calbindin, parvalbumin and secretigogin.

Suggestions for use: The antibody solution can be used be used at dilutions of 1:1,000 in immunofluorescence experiments. In western blotting using chemiluminescence, it can be used at dilutions of 1:1,000-1:5,000.

Storage Instructions: Shipped on ice. Please store at 4°C for regular uses. For long term storage, please leave frozen at -20°C and avoid freeze/thaw cycles.

Limitations: This product is for research use only and is not approved for use in humans or in clinical diagnosis.

References:

1: Rogers JH: Calretinin: a gene for a novel calcium-binding protein expressed principally in neurons. J Cell Biol 105:1343-1353 (1987).

2: Kretsinger RH & Nockolds CE. Carp Muscle Calcium-binding Protein: II. Structure determination and general description.J. Biol. Chem. 248:3313-3326 (1973).

3: Andressen C, Bliimcke I & Celio MR. Calcium-binding proteins: selective markers of nerve cells. Cell Tissue Res 271:181-208 (1993).

4: Schwaller B, Durussel I, Jermann D, Herrmann B, Cox JA: Comparison of the Ca2+-binding properties of human recombinant calretinin-22k and calretinin.J Biol Chem 272: 29663-29671 (1997).

5: Stevens J, Rogers JH: Chick calretinin: purification, composition, and metal binding activity of native and recombinant forms. Protein Expr Purif 9: 171-181, (1997).

6: Schiffmann SN, Cheron G, Lohof A, d'Alcantara P, Meyer M, Parmentier M, Schurmans S. Impaired motor coordination and Purkinje cell excitability in mice lacking calretinin. Proc Natl Acad Sci U S A. 27: 5257-62 (1999).