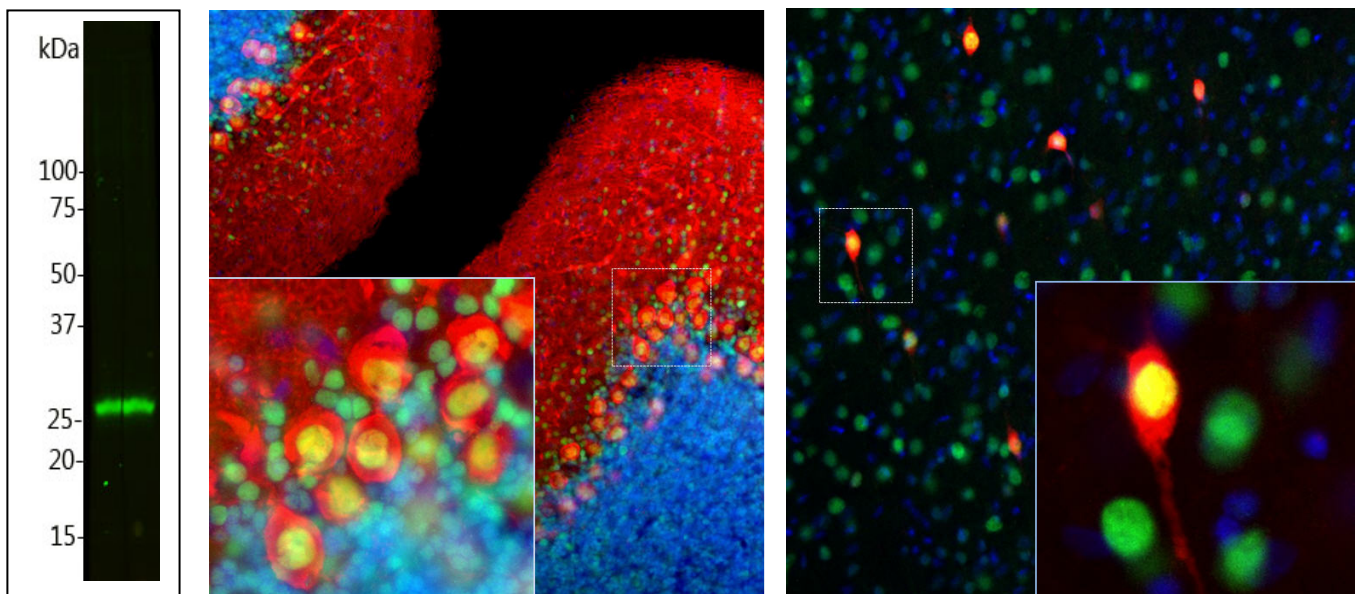


Catalogue# MCA-4H7: Monoclonal antibody to Calbindin

The Immunogen: Calbindin, also known as calbindin 1 or calbindin-D28k, is a member of the large superfamily of cytoplasmic Ca²⁺ binding proteins. Calbindin-1 belongs to the subclass of these protein families containing the "EF hand" Ca²⁺ binding motif originally characterized in parvalbumin (1). Calbindin is expressed in mammalian brain, intestine, kidney 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 (2). It is particularly concentrated in the dendrites and perikarya of cerebellar Purkinje cells, but is also found in many GABAergic interneurons in the cortex as well as striatum. These GABAergic interneurons in most cases express only one of the three Ca²⁺ binding proteins, namely calbindin, parvalbumin or calretinin. As a result, these important inhibitory interneurons can be identified and subclassified based on their content of these three proteins (2). 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 calbindin is a 261 amino acid protein with an apparent molecular weight of 30 kDa. It is related in primary sequence to calretinin, which is also known as 29 kDa calbindin and calbindin-2. The primary sequence and NMR structure of calbindin indicate six distinct Ca²⁺ binding sites corresponding to the EF hands motifs. Of the six sites, four bind Ca²⁺ with relatively high affinity. The function of calbindin-1 appears to be primarily buffering the Ca²⁺ level in cells. The affinity of calbindin for Ca²⁺ is low at the typical resting cytoplasmic Ca²⁺ level of around 100 nM, and the protein only binds Ca²⁺ significantly when the level increases greatly. Accordingly, it is widely thought that the primary function of this protein is to act as a Ca²⁺ buffer. Buffering Ca²⁺ is important, as uncontrolled increases in the level of this cation can lead to both apoptosis due to Ca²⁺ stimulated release of proteins from mitochondria and necrosis due to the activation of Ca²⁺ dependent proteases. Knockout of the calbindin-1 gene in mice leads to ataxia and other motor problems, consistent with the fact that a large amounts of this protein normally presents in the cerebellum (3). The HGNC name for this protein is CALB1.



Left: Western blot analysis of MCA-4H7. Blots of cow cerebellum lysate were probed with MCA-4H7. The MCA-4H7 monoclonal binds strongly and specifically to the calbindin band at 28kDa. Rat brain cerebellum (**Middle**) and cortex (**Right**) sections (45 μ M; fixed by transcardial perfusion with 4% paraformaldehyde) were co-stained with our MCA-4H7 (red) and anti-MeCP2 rabbit antibody (**RPCA-MeCP2**) (green). Calbindin is predominantly expressed in the dendrites and perikarya of Purkinje cells in the molecular layer of cerebellum, and selectively expressed in certain type of interneurons (calbindin-positive interneuron) in the cortex. MeCP2 is universally expressed in the nuclei of almost all neurons. As a result, calbindin-expressing cell soma is strongly

labeled with red, but nucleus appears to be yellow. Blue is Dapi nucleus staining. Insets are high magnification images of the boxed areas.

Antibody characteristics: MCA-4H7 is a mouse IgG1 class antibody. It was raised against the full-length recombinant Calbindin protein expressed in and purified from *E. coli*.

Suggestions for use: The antibody solution is affinity purified from tissue culture supernatant and is at concentration of 1 mg/mL in phosphate buffered saline with 50% glycerol. The antibody solution can be used at dilutions of at least 1:5,000 in immunofluorescence experiments. In western blotting using chemiluminescence it can be used at dilutions of 1:5,000. Antibody preparation contains 5 mM sodium azide preservative (Link to <http://www.encorbio.com/MSDS/azide.htm> for Material Safety Data Sheet).

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. Kretsinger RH, Nockolds CE. Carp Muscle Calcium-binding Protein: II. Structure determination and general description. *J. Biol. Chem.* 248:3313-3326 (1973).
2. Andressen C, Bliimcke I, Celio MR. Calcium-binding proteins: selective markers of nerve cells. *Cell Tissue Res* 271:181-208 (1993).
3. Schwaller B, Meyer M, Schiffmann S. 'New' functions for 'old' proteins: The role of the calcium binding proteins calbindin D-28k, calretinin and parvalbumin, in cerebellar physiology. Studies with knockout mice. *The Cerebellum* 1:241-258 (2002).