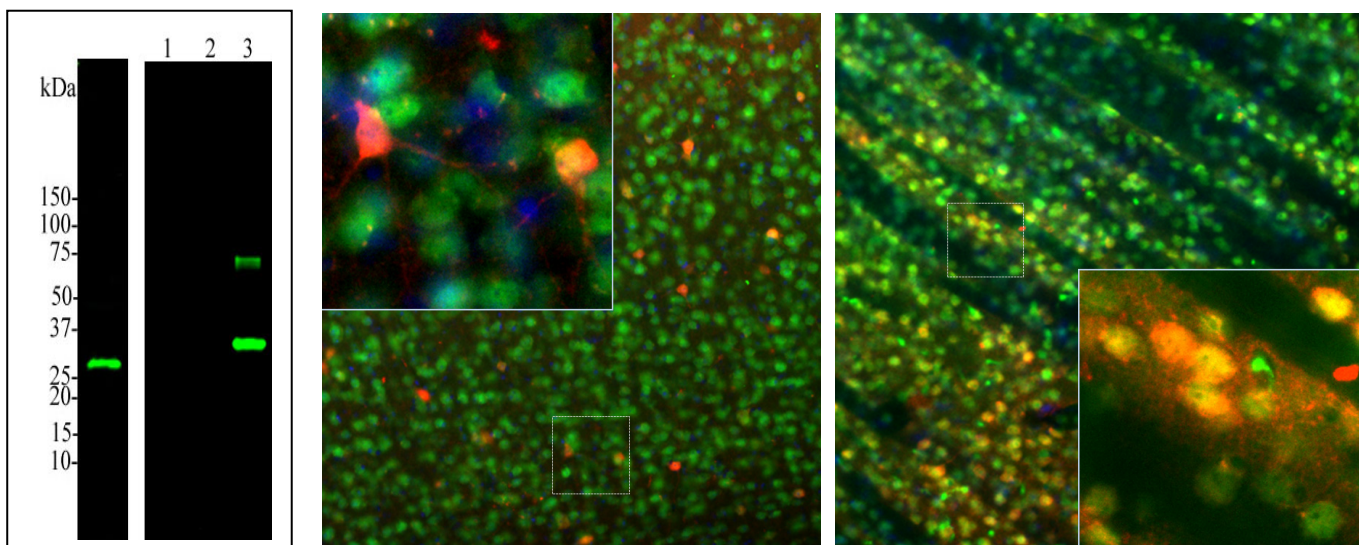


Catalogue# CPCA-Calb: Chicken Polyclonal Antibody to Calbindin

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 large amounts of this protein normally present in the cerebellum (3). The HGNC name for this protein is CALB1. Our CPCA-Calb antibody was raised against full-length recombinant human calbindin protein expressed in and purified from *E.coli*.



Left: Western blot analysis of CPCA-Calb. Blots of rat brain lysate (left), 0.5 µg of His-tagged recombinant proteins (right) were probed with CPCA-Calbindin at 1:5,000. Lane1: Parvalbumin, Lane 2: Calretinin, Lane 3: Calbindin. In rat brain lysates, this antibody recognizes a clear band at ~30 kDa which represents calbindin, and it reacts only with calbindin protein (Lane3) but not other calcium-binding proteins (Lane1 and 2). The band at ~60 kDa is most likely the dimer of calbindin. **Middle and Right:** Adult rat brain cortex (**Middle**) and striatum (**Right**) sections (45 µM; fixed by transcardial perfusion with 4% paraformaldehyde) were stained with our

CPCA-Calbindin (1:1,000, red), and our monoclonal mouse anti-Fox3/NeuN (**MCA-1B7**; green). Calbindin labels a subset of sparsely-distributed interneurons (calbindin-positvie interneurons) in the cortex (Middel), and more densely-distributed neurons in the striatum (Right). Since neurons also express Fox3/NeuN, calbindin-positive cells appear to be gold to yellow. Insets are high magnification images of boxed area of each image. Blue is Dapi nucleus staining.

Antibody Characteristics: This antibody was generated in chicken by standard procedures and immunoglobulin was extracted from egg yolk. The resulting polyclonal antibody belongs to the IgY subclass. This is the chicken homologue of mammalian IgG and can be used in the same general way, with the caveat that this type of antibody does not bind either Protein A or Protein G. Suitable secondary antibody reagents can be obtained from many vendors including Molecular Probes and Sigma-Aldrich.

Suggestions for use: The IgY solution is at a concentration of ~10 mg/mL. It can be used at dilutions of 1:1,000-1:5,000 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. 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 \(2002\)](#).