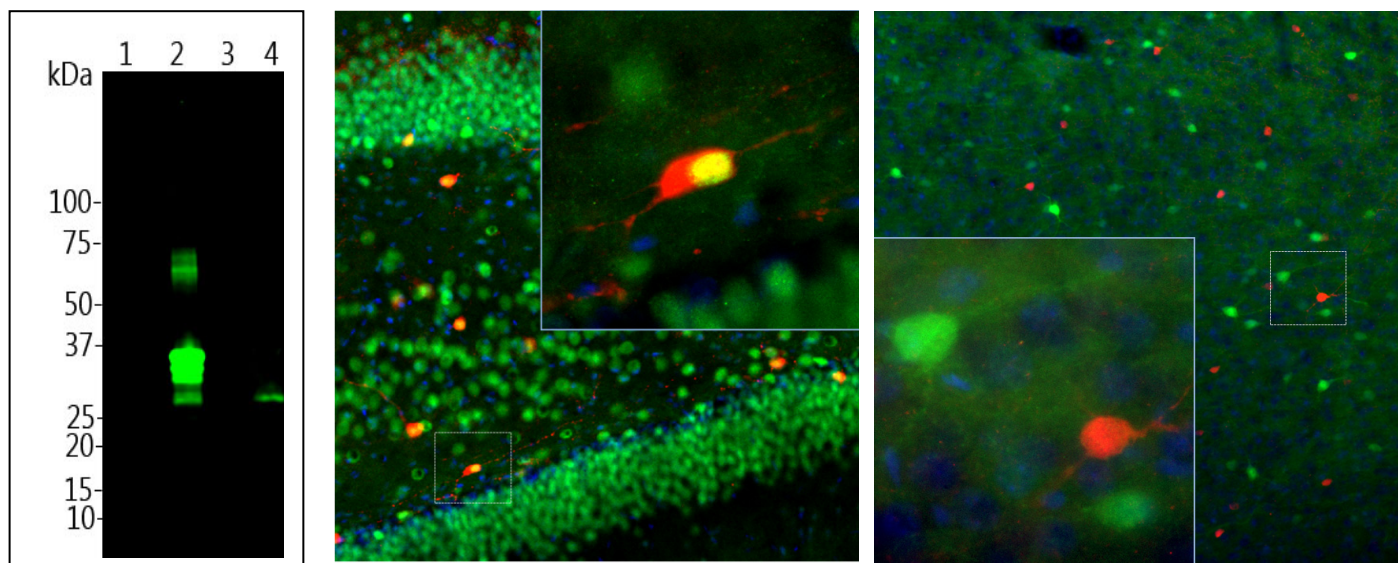


Catalogue# CPCA-Calretinin: Chicken Polyclonal Antibody to Calretinin

The Immunogen: Calretinin, first described in 1987, acquired its name based on homology with calcium binding protein calbindin and the tissue of first detection, the chick retina (1). As a member of the large superfamily of cytoplasmic Ca^{2+} binding proteins, calretinin belongs to the subclass of proteins 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 some cerebellar granular cells and their parallel fibers, but is also found in many GABAergic interneurons in the cortex. These GABAergic interneurons, in most cases, express only one of three Ca^{2+} 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 Ca^{2+} with high affinity in a cooperative manner, one with low affinity and the last one is non-functional, without Ca^{2+} -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 the dentate gyrus and granule cells results in 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.

This antibody 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 calbindin and parvalbumin in Western and immunostaining.



Left: Western blot of recombinant protein parvalbumin (lane 1), calretinin (lane 2), calbindin (lane 3) and rat brain lysates (lane 4) was probed with CPCA-Calretinin (1:1,000). In rat brain lysates, this antibody recognizes a clear band at ~31 kDa and it reacts only with calretinin protein, and not other calcium-binding proteins. **Middle:** Adult rat brain hippocampus section (45 μM ; fixed by transcardial perfusion with 4% paraformaldehyde) was stained with CPCA-Calretinin (1:1,000, red), and our rabbit anti-MeCP2 (**RPCA-MeCP2**; green). Calretinin labels a subset of hippocampal interneurons, which also express MeCP2 in the nucleus to give a yellow color. **Right:** Adult rat cortex section was co-stained with CPCA-Calretinin (red) and our mouse anti-calbindin antibody (**MCA-4H7**; green). Each antibody specifically labels a subset of interneurons (i.e., calretinin-positive or calbindin-positive) that express each marker exclusively. Insets are high-magnification images of the boxed area in each picture. Blue is Dapi staining that labels DNA.

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: 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).