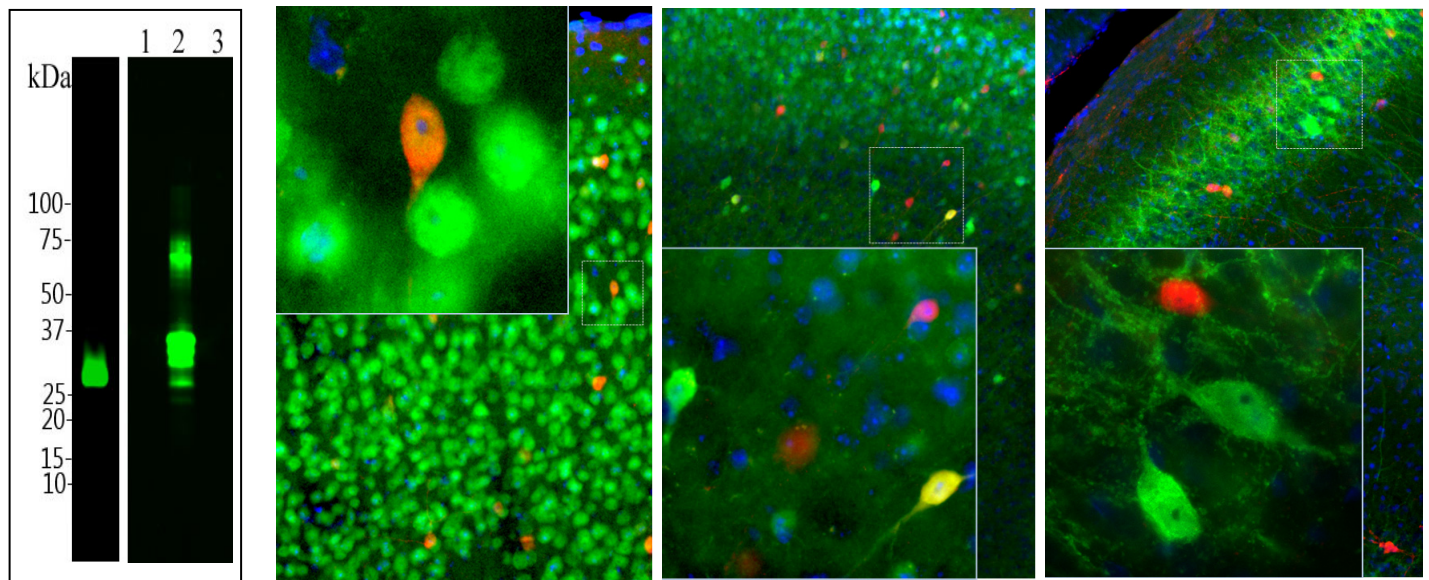


Catalogue# RPCA-Calretinin: Rabbit Polyclonal Antibody to Calretinin

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 Ca²⁺ binding proteins, calretinin belongs to the subclass of these containing the "EF hand" Ca²⁺ 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 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 Ca²⁺ 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 (see Blot image).



Left: Blot of 20µg of rat brain lysates (left) and blot of 0.2µg of recombinant protein (right): parvalbumin (lane 1), calretinin (lane 2), calbindin (lane 3) were probed with RPCA-Calretinin at 1:5,000. In rat brain lysates, this antibody recognizes a clear band at ~29 kDa and it reacts only with calretinin protein, and not other calcium-binding proteins. **Middle Left:** Adult mouse brain section (45 µM; fixed by transcardial perfusion with 4% paraformaldehyde) across motor cortex was co-stained with RPCA-Calretinin (red) and our mouse monoclonal antibody to Fox3/NeuN (**MCA-1B7**; green). **Middle Right:** Adult mouse brain section across visual cortex was co-stained with RPCA-Calretinin (red) and our chicken polyclonal antibody to calbindin (**CPCA-Calb**; green). Calretinin and calbindin label different population of neurons in the brain. As a result, most cells were labeled with one of the two antibodies and appear to be either red or green. However in visual cortex, a few cells express both proteins and appear to be yellow. **Right:** Adult rat brain section (45 µM; fixed by transcardial perfusion with 4% paraformaldehyde) across hippocampal CA1 region was co-stained with RPCA-Calretinin (red) and our mouse monoclonal antibody to parvalbumin (**MCA-3C9**; green). The two antibodies stain distinct subsets of interneurons in the pyramidal layer and the positively labeled cells

appear to be either red or green. Insets show high magnification pictures of boxed area in each image. Blue is a Hoechst staining that labels DNA.

Antibody Characteristics: This antibody was generated in rabbit by standard procedures. Store at 4°C or -20°C. Avoid repeat freezing and thawing.

Suggestions for use: Antibody can be used at dilutions of 1:5,000-1:10,000 in immunofluorescence experiments. In western blotting using chemiluminescence, it can be used at dilutions of 1:5,000-1:10,000.

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).
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- 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).