



Catalog Number:	MO22165	Host:	Mouse
Product Type:	Monoclonal IgG1 Affinity Purified Antibody	Species Reactivity:	Human, rat, mouse, cow
Immunogen Sequence:	Full length recombinant human protein HGNC name for this protein is CALB2	Format:	Liquid, 100 ul aliquot of 1 mg/ml concentration diluted at 50% glycerol/PBS
Applications:	Immunofluorescence/Immunocytochemistry: 1:1,000 Immunohistochemistry: 1:1,000 Western Blot: 1:1,000-1:5,000		
	Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Antibody can also be aliquoted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Avoid repeated freeze-thaw cycles.		

Application Notes

Description/Data:

Calretinin is a member of the large superfamily of cytoplasmic Ca²⁺ binding proteins, thus belongs to the subclass of these containing the "EF hand" Ca²⁺ binding motif originally characterized in parvalbumin. 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. 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. 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.

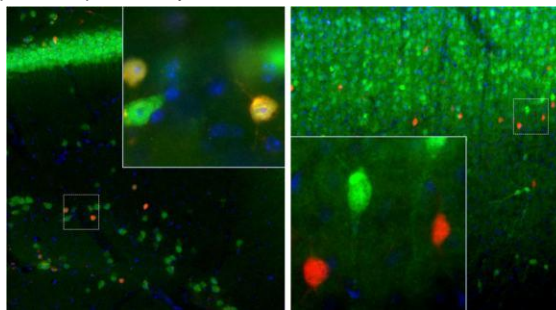
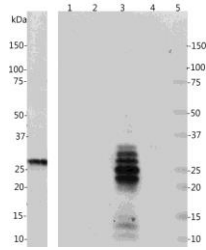


Image: Left: Adult mouse brain hippocampal section (45 μM; fixed by transcardial perfusion with 4% paraformaldehyde) was co-stained with Calretinin MO22165 (1:1000, red) and our rabbit polyclonal antibody to Fox3/NeuN (RA22113, green). Calretinin stains a small number of interneuron in the stratum radiatum 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 MO22165 (red) and our chicken polyclonal antibody to calbindin (CH22118, 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.

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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. 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. The HGNC name for this protein is CALB2.

Image: Western blot analysis of MO22165. **Left:** Blot of cow cerebellum lysate was probed with MO22165. The MO22165 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 MO22165. Lane 5 is protein marker. This antibody recognizes only calretinin, not other calcium-binding proteins.

Immunostaining of cells in tissue culture:

The purpose of fixation is denaturing the components of cells enough so that they stay on the dish and can be bound by antibodies, hopefully without destroying cellular morphology. Fixatives such as formalin, paraformaldehyde and glutaraldehyde chemically cross-link proteins, by binding to amino acid side chains, generally the most chemically reactive ones like amines (Lysine, Arginine, Glutamine and Asparagine). This chemical modification can also have the consequence of blocking antibody binding sites. Substances such as acetone and methanol are not true fixatives but are denaturants, which precipitate proteins without covalently modifying them. We routinely use a combination of mild formalin fixation followed by cold methanol for neurons, mixed neuron/glial cultures and most of the widely used human and rodent cell lines. The formalin preserves the cellular morphology quite well, while the methanol further denatures the proteins of the cells and helps keep what is left of the cell adherent to the dish. For soluble proteins it may be necessary to miss the methanol step, but in this case you have to be very careful with the washing steps, as the cells tend to wash off the dish. Certain antibodies may be very sensitive to formalin fixation, so you may have to experiment a little, perhaps missing out that step. The following procedure works for antibodies to most cytoskeletal and signaling molecules. This procedure is good for cells in 6 well tissue culture plates or in 35mm tissue culture dishes. These are just big enough that you can look from above with a typical immunofluorescence microscope through a glass coverslip. This allows you to see the specimens very well and take very high quality pictures. (However note that it's a pain to change lenses on the microscope if you use the 6 well dishes, since you have to rack the lens right the way up to do this, and you have to take out the two neighboring lenses in the turret since they will hit the other wells of the dish! It's less of a problem with 35mm dishes but still a pain. No procedure is perfect....).

1. Draw off culture medium with aspirator and add 1 mL of 3.7 % formalin in PBS solution to the dish. (make up from 10 mLs Fisher 37% formalin plus 90mLs PBS, the Fisher formalin contains 37% formaldehyde plus about 1% methanol which may be relevant sometimes). Let sit at room temp for 1 minute. (can add 0.1% Tween 20 to PBS used here and all subsequent steps to reduce background; probably best not to do this first time round though as it may extract your antigen or help wash your cells off the dish).
2. Take off the formalin/PBS and add 1ml of cold methanol (-20°C, kept in well-sealed bottle in fridge). Let sit for no more than 1 minute.
3. Take off methanol and add 1ml of PBS, not letting the specimen dry out. To block nonspecific antibody binding can add ~10 μL (=1%) of goat serum (Sigma), and can incubate for 30 minutes. Can then add antibody reagents. Typically, 100 μL of hybridoma tissue culture supernatant or 1ml of mouse ascites fluid or crude serum. Incubate for 1 hour at room temp. (or can go at 37°C for 30 minutes to 1 hour, or can do 4°C overnight, exact time not too critical). Can do very gentle shaking for well adherent cell lines (3T3, Hek293 etc.).
4. Remove primary antibody and replace with 1 mL of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.
5. Add 0.5 μL s of secondary antibody. These are fluorescently labeled Goat anti mouse or rabbit antibodies and are conjugated to ALEXA dyes and were originally marketed by Molecular Probes (Eugene Oregon, the ALEXA dyes are sulphonated rhodamine compounds and are much more stable to UV than FITC, TRITC, Texas red etc. Molecular Probes was bought by Invitrogen, which now markets these reagents). Typically make 1:2,000 dilutions of these secondaries in PBS plus 1% goat serum, BSA or non fat milk carrier. Incubate for 1 hour at room temp. (or can go at 37°C for 30 minutes to 1 hour, or can do 4°C overnight). Can do gentle shaking for well adherent cell lines (3T3, HEK293 etc.).
6. Remove secondary antibody and replace with 1 ml of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.
7. Drop on one drop of Fisher mounting medium onto dish and apply 22 mm square coverslip. View in the microscope!

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