NEUROMICS

Parvalbumin Data Sheet

Catalog Number:	MO22149	**Host:**	Mouse
Product Type:	Monoclonal IgG1 Affinity Purified Antibody	**Species Reactivity:**	Human, rat, mouse, cow
Immunogen Sequence:	Full length recombinant protein. HGNC name for this protein is PVALB	**Format:**	Liquid, 100 ul aliquot Concentration: 1 mg/ml
Applications:	Immunofluorescence/Immunocytochemistry: 1:1,000-5,000 Immunohistochemistry: 1:1,00-5,000 Western Blot: 1:1,000-5,000 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:

Parvalbumin is a cytoplasmic low molecular weight Ca^{2+} binding proteins with. It is the prototypic member of the very large family of proteins containing the "EF hand" Ca^{2+} binding motif. The nomenclature comes from the parvalbumin structure in which the fifth and sixth alpha helices, the E and F helices, form a V shape including acid amino acids which co-ordinate a single Ca^{2+}. It turns out that close variants of this structure are found in many other Ca2+ binding proteins. Parvalbumin is expressed in fast-contracting muscles, where its levels are highest, as well as in the brain and some endocrine tissues. In brain, it is particularly concentrated in Purkinje cells and interneurons in the molecular layer of the cerebellum, 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 parvalbumin, calretinin, or calbindin. 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.
Parvalbumin contains 3 EF-hand domains, domain AB, CD and EF. The N-terminal EF-hand of parvalbumin does not bind Ca^{2+}, so that functional Ca2+ binding is between helices C and D and between helices E and F. The function of parvalbumin appears to be primarily buffering the Ca^{2+} level in cells. Absence of parvalbumin and calbindin disrupts the regulation of Purkinje cell firing rate and rhythmicity *in vivo* and parvalbumin dysfunction in cells critically contributes to abnormalities in oscillatory rhythms and network. The HGNC name for this protein is PVALB.

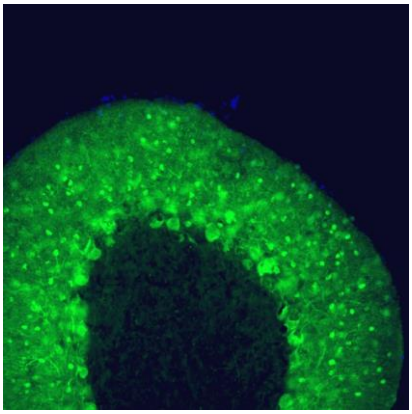

Image: Adult rat cerebellum floating section was stained with MO22149 at 1:1,000 to parvalbumin in green. Parvalbumin is prominently expressed in the dendrites and perikarya of Purkinje cells and some interneurons in the molecular layer. Blue is a DNA stain.

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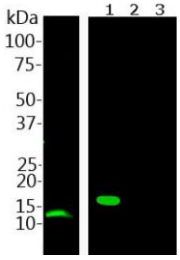
Image: Western blot analysis of MO22149 in rat skeletal muscle lysate (left) and His-tagged recombinant proteins (right): parvalbumin (lane 1), calretinin (lane 2) and calbindin (lane 3). Blots were probed with MO22149 at 1:1,000. In skeletal muscle lysates, this antibody recognizes a band at 12 kDa which represents parvalbumin. It reacts to recombinant parvalbumin only, not the other calcium-binding proteins listed. Note, this antibody does not recognize parvabumin in rat or mouse brain lysates on western blots. Protein from rat skeletal muscle lysates were transferred to PVDF membrane.



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 changes 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 of 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 supernatent 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 µLs 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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