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Product Description

Monoclonal anti Calretinin

CR 6B3

Concentrated supernatant

Product: Monoclonal anti-Calretinin

Code No: CR6B3

Form: Lyophilized concentrated supernatant (no preservatives).

Quantities available: 200 µl, 500 µl, and 1 ml

Reconstitution: with corresponding amount of bi-distilled water

Description:

The antibody 6B3 against calretinin was produced in mice by immunization with recombinant human calretinin-22k (1, 2). Calretinin 22k is an alternative splice product of the calretinin gene (3) and identical with calretinin up to Arg178. After fusion, hybridoma cells were screened with human recombinant calretinin as target and the clone 6B3 was selected. The antibody 6B3 recognizes an epitope within the first 4 EF-hands domains common to both calretinin and calretinin-22k (2). It was evaluated for specificity and potency: a) by Biotin-Avidin labeling of cryostat-, vibratome- and paraffin-sections of 4% paraformaldehyde (or 10% buffered formalin) fixed brains b) by immune-enzymatic labelling of immunoblots c) by immunohistochemistry on tissue calretinin knock-out mice (6)

The product is a monoclonal antibody against calretinin (4), a calcium-binding protein of the EF-hand family related to calbindin D-28k and calmodulin. The antibody reacts specifically with calretinin in tissue originating from various species (see immunoblot). This antibody does not cross-react with calbindin D-28k or other known calcium binding-proteins, as determined by its distribution in the brain, as well as by immunoblots. Calretinin is an attractive neuroanatomical tool and this McAB guarantees the continued production of a constant titer of anti-calretinin antibody with the same specificity and chemical identity. The antibody recognizes calretinin in human mesotheliomas (4,5).

Storage:

After reconstitution freeze in small aliquots (e.g. 1 µl) and keep at - 80°C (or at least - 20°C). For continuous use, keep at 4°C (with 0.01% Na-azide). Avoid repeated freezing and thawing.

Immunoblot of brain extracts

Antibody 6B3 specifically stains the calretinin band on immunoblots of brain extracts (Fig. 1).

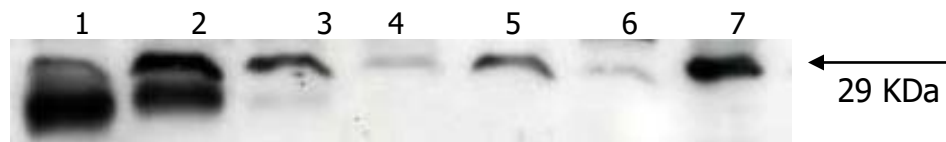


Fig. 1 Immunoblot of brain homogenate of various species with the monoclonal antibody 6B3.

1: Mouse; 2: Rat; 3: Guinea pig; 4: Rabbit; 5: Macaca fascicularis; 6: Zebrafish; 7: Chicken. In all species, a band at 29 KDa is detected.

Immunohistochemistry on calretinin knock out mice

Antibody 6B3 immunolabels a subpopulation of neurons in the normal brain with high efficiency (Fig. 2a), but does not stain the brain of calretinin knock out mice (Fig. 2b).

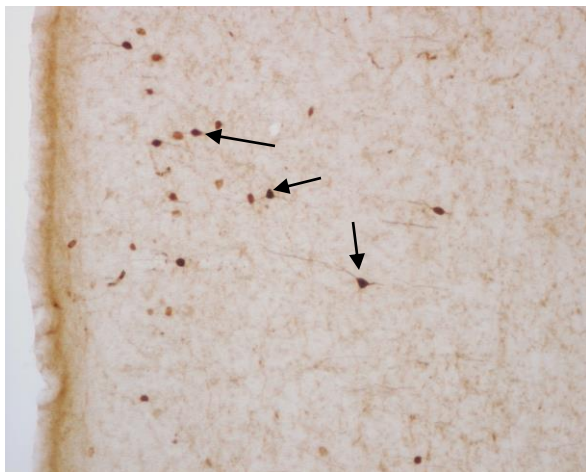


Fig 2a: Immunohistochemical staining with McAB 6B3 in the cerebral cortex of a control mouse. Notice the strong staining of scattered interneurons in the upper layers (arrows). X100



Fig 2b: Absence of specific immunohistochemical staining with McAB 6B3 in the cerebral cortex of a calretinin knock out mouse (5). Non-specific staining of the vessel walls (asterisk) due to the anti-mouse secondary antibody. X 100

Working dilutions:

Immunohistochemistry: 1:2'000 - 1:5'000, when performed on cryostat or paraffin-sections of paraformaldehyde (4%) or formalin-fixed tissue.

Immunoblots: 1:1'000 - 1:2'000.

For immunohistochemistry and immunoblots the titre was determined by using the avidin-biotin method. We recommend that the optimal dilutions be determined by titration experiments

References

1. Schwaller B., Buchwald P., Blümcke I., Celio M.R. and Hunziker W. (1994) Characterization of a polyclonal antiserum against the purified human recombinant calcium-binding protein calretinin. *Cell Calcium* 14: 639-648.
2. Zimmermann, L. and Schwaller B (2002) Monoclonal antibodies recognize epitopes of calretinin. *Cell Calcium* 31: 13-25.
3. Rogers J.H. (1987) Calretinin: a gene for a novel calcium-binding protein expressed principally in neurons. *J. Cell Biol.* 105:1343-1353.
4. Gotzios V., Vogt P. and M.R. Celio (1995) Calretinin is a selective marker for malignant pleural mesotheliomas of the epithelial type. *Pathol. Res. Pract.* 192:137-147.
5. Doglioni, C. et al. (1996) Calretinin: a novel immunocytochemical marker for mesothelioma. *Am. J. Surg. Pathol.* 20:1037-1046.
6. Schiffmann S.N. et al (1999) Impaired motor coordination and Purkinje cell excitability in mice lacking calretinin. *PNAS*, 96: 5257-5262.