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Product Description

CR 7699/3H

Rabbit anti Calretinin

Product : Rabbit anti-calretinin

Code No : 7699/3H

Lot no: 18299

Form : Lyophilized whole serum (no preservatives).

Quantity: 200 µl.

Reconstitution: with 200 µl of bidistilled water.

Description

The antiserum against calretinin is produced in rabbits by immunization with recombinant human calretinin containing a 6-his tag at the N-terminal. The antibody was evaluated for specificity and potency: a) by Biotin-Avidin labeling of cryostat-, vibratome- and paraffin-sections of 4% paraformaldehyde fixed brains and b) by immunoenzymatic labelling of immunoblots.

The product is a polyclonal antiserum against calretinin (1), 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 human, monkey, rat, mouse, guinea pig, chicken and fish (Fig. 1). This antiserum does not cross-react with calbindin D-28k or other known calcium binding-proteins, as determined by immunoblots and by its distribution in the brain (Fig. 2).

Immunoblot

In Fig. 1, extracts of soluble proteins were isolated from whole brains of different species and separated by SDS-PAGE. In the Western blot the antiserum 7699/3H specifically recognizes a band of 29-30k Da.



Fig. 1 Immunoblot of brain homogenates of various species with antiserum 7699/3H. 1: Mouse, 2: Rat, 3. Guinea pig, 4. Rabbit, 5. Macaca fascicularis, 6. Zebrafish, 7. Chicken, 8. recombinant calretinin

Immunohistochemistry on Calretinin knock-out mice

Antibody 7699/3H labels 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 antiserum 7699/3H in the cerebral cortex of a control mouse. Notice the staining of scattered interneurons in various layers. X100



Fig 2b: Absence of specific immunohistochemical staining with antiserum 7699/3H in the cerebral cortex of a calretinin knock-out mouse (2). X 100

Uses

Calretinin belong probably to the class of "trigger" calcium binding-proteins. It occurs mainly in subpopulations of nerve cells and is an excellent markers for mesotheliomas (3,4).

Working dilutions

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

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

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

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.

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. Schiffmann S.N. et al (1999) Impaired motor coordination and Purkinje cell excitability in mice lacking calretinin. *PNAS*, 96: 5257-5262.
3. Gotzos 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.
4. Doglioni, C. et al. (1996) Calretinin: a novel immunocytochemical marker for mesothelioma. *Am. J. Surg. Pathol.* 20:1037-1046.