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# Product Description Monoclonal anti-

Calbindin

**CB300** 

Concentrated supernatant

Product: Monoclonal anti Calbindin D-28k

Code No.: CB300

Form: Lyophilized concentrated supernatant (no preservatives).

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

Reconstitution: with corresponding amount of bi-distilled water

#### **Description:**

Monoclonal anti-calbindin D-28k 300 is a mouse IgG1 produced by hybridization of mouse myeloma cells with spleen cells from mice immunized with calbindin D-28k purified from chicken gut (1). The antibody was evaluated for specificity and potency: a) by indirect immunofluorescence or immunoperoxidase labelling as well as Biotin-Avidin labeling of cryostate or vibratome-sections of 4 % paraformaldehyde fixed tissue; b) by immunoenzymatic labelling of immunoblots; c) by radioimmunoassay.

The product is a monoclonal antibody (McAB) against calbindin D-28k, a calcium-binding protein of the EF-hand family related to calmodulin and troponin-C.

McAB 300 reacts specifically with calbindin D-28k on immunoblots of extracts of tissue originating from human, monkey, guinea pig, rabbit, rat, mouse and chicken (but probably not fish, Fig. 1). McAB 300 does not cross-react with calretinin or other known calcium binding-proteins. McAB 300 specifically stains the <sup>45</sup>Ca-binding spot of calbindin D-28k (MW 28'000, IEP 4.8) in a two-dimensional gel. In radioimmunoassay it detects calbindin D-28k with a sensitivity of 10 ng/assay and an affinity of 1.6 x 10<sup>12</sup> L/M.

McAB 300 against calbindin D-28k specifically localizes calbindin using free-floating or mounted sections of brain, kidney and pancreas of probably all vertebrates with the exception of fishes.

Calbindin D-28k is an attractive neuroanatomical tool and this McAB guarantees the continued production of a constant titer of anti-calbindin D-28k antibody with the same specificity and chemical identity.

### Immunoblot of brain extracts:

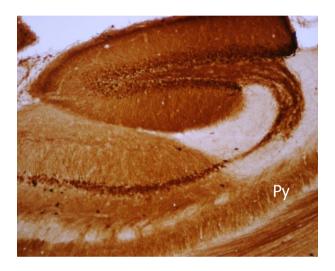
1 2 3 4 5 6

— ← 28 Kda

Fig. 1 Immunoblot of brain homogenate of various species with the monoclonal antibody CB300 1: Mouse; 2: Rat; 3. Guinea pig; 4. Rabbit; 5. *Macaca fascicularis*; 6. Zebrafish; 7. recombinant calbindin D-28k. In all species (except fish) only a band at 28 kDa is detected.

# Immunohistochemistry on Calbindin D-28k knock out mice

Antibody CB300 immunolabels a subpopulation of neurons in the brain with high efficiency (Fig. 2a) but does not stain in the brain of calbindin D-28k knock out mice (Fig. 2).



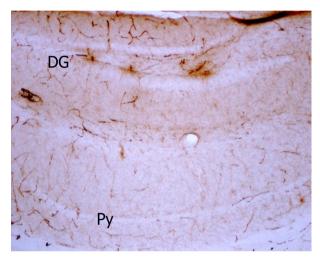


Fig 2a: Immunohistochemical staining with McAB300 in the hippocampus of a control mouse. Notice the strong staining of the dentate gyrus (DG) and of the pyramidal cells (Py) of the hippocampus. X100

Fig 2b: Lack of specific immunohistochemical staining with McAB 300 in the hippocampus of a Calbindin D-28k knock out mouse (6). Non-specific staining of the vessel walls due to the anti-mouse secondary antibody. DG: dentate gyrus, Py: pyramidal cells X 100

## **Working dilution:**

Immunohistochemistry: 1:3'000 - 1:10'000, when performed with the avidin-biotin method. Immunoblots: 1:100 - 1:1'000 with an indirect peroxidase method. We recommend that the optimal dilutions be determined by titration experiments.

#### Storage:

For long storage, 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. Celio M.R. et al., Cell Calcium 11:599-602, 1990
- 2. Kretsinger R.H. (1981) Neurosci. Res. Progr. Bull. 19/8, MIT-Press
- 3. Garcia-Segura L.M. et al. (1984) Brain Res. 296: 75-86.
- 4. Airaksinen M.S., et al, (1997), PNAS 94(4): 1488-1493