

Product Information

ANTI-CALBINDIN-D-28K (KD-15)

Developed in Rabbit, Affinity Isolated Antibody

Product Number **C 7354**

Product Description

Anti-Calbindin-D-28K (KD-15) is developed in rabbit using a synthetic peptide corresponding to the C-terminal region of rat calbindin-D-28K (amino acids 185-199) conjugated to KLH as immunogen. This sequence is specific for calbindin-D-28K and is not found in other members of the EF-hand family such as calbindin-D-9K, calretinin, myosin light chain, parvalbumin, S-100a, S-100b, S100A2 (S100L) and S100A6 (calcyclin). This sequence is identical in the corresponding human, mouse and bovine calbindin-D-28K sequences and is highly conserved (single amino acid substitution) in chicken and frog calbindin-D-28K. Anti-Calbindin-D-28K (KD-15) is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-Calbindin-D-28K (KD-15) recognizes human, rat and chicken calbindin-D-28K (28 kDa). Due to the similarity between species, reactivity with mouse, bovine and frog is expected, but has not been confirmed. Applications include immunoblotting and immunohistochemistry (formalin-fixed, paraffin-embedded tissue sections). The antibody does not cross-react with calretinin (human). By immunoblotting, staining of calbindin-D-28K is specifically inhibited with the calbindin-D-28K immunizing peptide (rat, amino acids 185-199). Enzymatic predigestion of formalin-fixed, paraffin-embedded sections by proteolytic enzymes (e.g., 0.1% trypsin or protease, 10 min., at RT or 37 °C) improves immunohistochemical staining with the antibody.

Calbindin-D-28K (also termed vitamin D-dependent calcium-binding protein, or cholecalciferin),^{1,2} is a highly conserved 28 kDa calcium binding protein, with broad tissue distribution.³ It belongs, together with calmodulin, S-100, parvalbumin, troponin C and other proteins, to a family of low molecular weight calcium-binding proteins (CaBPs). These CaBPs have homologous primary structures, which contain conserved polypeptide folds of the EF-hand type for Ca²⁺ binding. There are two types of CaBPs: the “trigger”- and the “buffer”-CaBPs. The “trigger” type CaBPs act by changing their conformation upon Ca²⁺ binding. This distortion exposes regions on the surface of the protein, which interact with target molecules, thus altering their

activity. The CaBPs of the “buffer” type are thought to control the intracellular calcium concentration. Ca²⁺ acts as a secondary messenger conveying extracellular signals and is involved in the regulation of multiple cell functions. In the central nervous system (CNS), Ca²⁺ plays a central role in synaptic transmission and axonal transport and both mechanisms require the presence of specific CaBPs that exert regulatory functions.⁴ Calbindin-D-28K is an important neuroanatomical marker. Despite its broad tissue distribution, it exhibits a cell-type-specific expression pattern. It has been immunocytochemically localized in selected cells in many CNS structures, where it is thought to buffer intracellular calcium, or transport intramembranous calcium. Calbindin-D-28K is found predominantly in subpopulations of central and peripheral nervous system neurons, and in certain epithelial cells involved in Ca²⁺ transport such as distal tubular cells and cortical collecting tubules of the kidney, and in enteric neuroendocrine cells.^{5,6}

Reagents

Anti-Calbindin-D-28K (KD-15) supplied as an affinity isolated antibody in 10 mM phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

Store at 2 °C to 8 °C for up to one month.

For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in “frost-free” freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

A minimum working dilution of 1:1,000 is determined by immunoblotting using recombinant rat calbindin-D-28K.

A minimum working dilution of 1:500 is determined by indirect immunoperoxidase staining of formalin-fixed, paraffin-embedded rat, human or chicken cerebellum sections.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilution by titration test.

References

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3. Garcia-Segura, L.M., et al., Brain Res., **296**, 75 (1984)
4. Chard, P.S., et al., Proc. Natl. Acad. Sci. USA, **92**, 5144 (1995).
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6. Ohm, T.G., et al., Neuroscience, **42**, 823 (1991).

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