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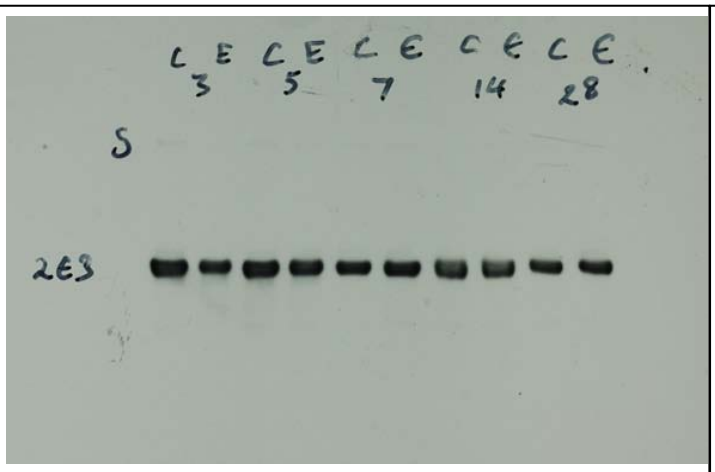
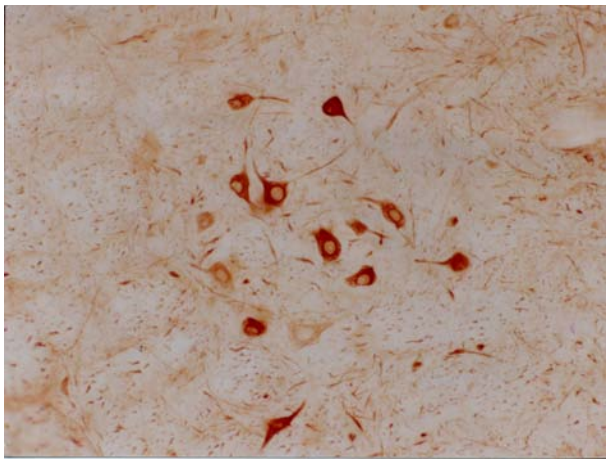
Catalogue# MCA-2E3: α-Internexin / NF66 Monoclonal Antibody clone 2E3

The Immunogen: α-internexin is a Class IV intermediate filament originally discovered as it copurifies with other neurofilament subunits (1). On SDS-PAGE gels it runs with an apparent molecular weight of 64 to 66 kDa, with some species variability, although the real molecular weight is about 55 kDa; as with the other neurofilament subunits the presence of highly negatively charged sequences results in reduction of SDS-PAGE mobility. α-internexin is related to but distinct from the better known neurofilament triplet proteins, NF-L, NF-M and NF-H, having similar protein sequence motifs and a similar intron organization. It is expressed only in neurons and in large amounts early in neuronal development, but is down-regulated in many neurons as development proceeds. Many classes of mature neurons contain α-internexin in addition to NF-L, NF-M and NF-H. In some mature neurons α-internexin is the only neurofilament subunit expressed. Antibodies to α-internexin are therefore unique probes to study and classify neuronal types and follow their processes in sections and in tissue culture. In addition the very early developmental expression of α-internexin means its presence is an early and convenient diagnostic feature of neuronal progenitors cells and other cell committed to the neuronal lineage. In addition recent studies show a marked up-regulation of a-internexin during neuronal regeneration (2). The use of antibodies to this protein in the study of brain tumors has not been examined to date, but is likely to be of interest.

Recently Trojanowski, Lee and coworkers used this antibody to show that α-internexin is an abundant component of the inclusions of neurofilament inclusion body disease (NFID, see two Cairns et al. papers listed below), a serious human neurodegenerative disorder. The antibody was also used to confirm the presence of circulating auto-antibodies to α-internexin in the sera of some patients with endocrine autoimmunity, as well as in some normal individuals (Rajasalu et al., 2004, listed below). The HGNC name for this protein is INA.



Crude homogenates of rat facial nucleus were run out on 7.5% SDS-PAGE gels and electrophoretically transferred to nitrocellulose. The nitrocellulose membranes were then blotted with MCA-2E3 monoclonal antibody. Note the strong clean ~66 kDa band and complete absence of any other bands, despite the presence of virtually all rat brain proteins on the membrane.



Section of adult rat brain stem showing facial nucleus 3 days after lesion of the contralateral facial nerve. Stained using ABC-DAB technique with MCA-2E3 monoclonal antibody to α-internexin. Note strong cytoplasmic staining of the neuronal cell bodies which are going through a regenerative response (2).

Antibody characteristics: MCA-2E3 was raised against recombinant rat α-internexin expressed in and purified from *E. coli* (3). MCA-2E3 reacts with human α-internexin and with this protein in all mammalian species tested to date. On Western blots, the antibody is unusually strong and specific in binding, see above. Can be used on formalin-fixed cells in tissue culture, cryostat sections, and Western blotting. The epitope recognized by the 2E3 clone is in the C-terminal non-helical extension of the protein and is unusually resistant to aldehyde fixation, so that this antibody is ideal for studies of paraffin embedded formalin fixed histological sections (see below). The antibody preparation is derived from CL350 bioreactors and contains about 5% calf serum. The antibody concentration, estimated from SDS-PAGE, is about

0.5 mg/mL We supply 0.5mL aliquots of this material. The material contains 10 mM sodium azide as a preservative (link to http://www.encorbio.com/MSDS/azide.htm for a material safety data sheet (MSDS).

Suggestions for use: For immunofluorescence try dilutions of 1:500, for immunohistochemistry with ABC or other enzymatic amplification procedures try 1:5,000. For western blots try 1:10,000 dilutions.

Limitations: This product is for research use only and is not approved for use in humans or in clinical diagnosis.

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Recent Papers using this antibody:

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