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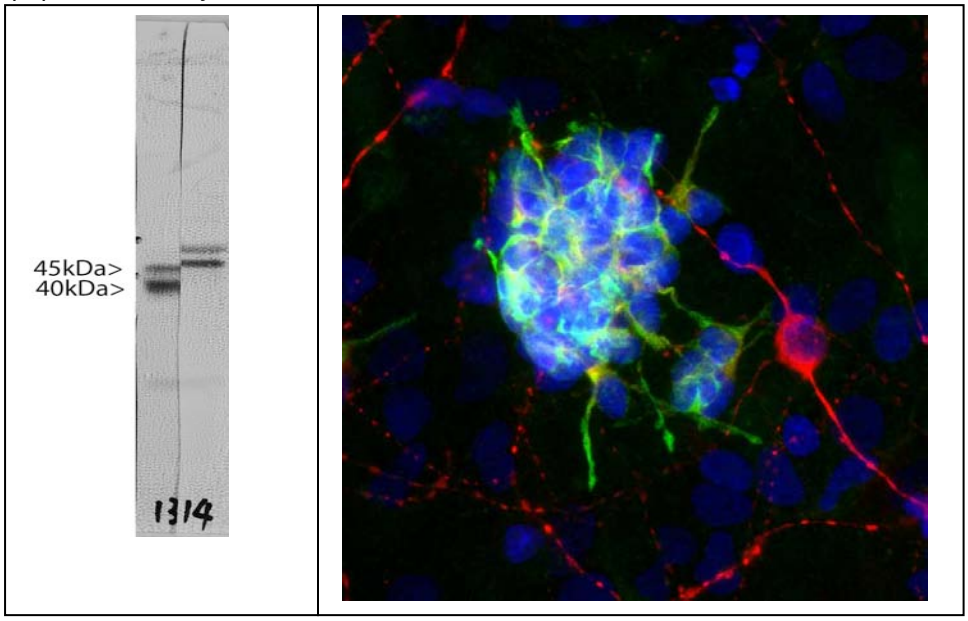
Catalogue# MCA-3E1: Mouse Monoclonal Antibody to Doublecortin/DCX

The Immunogen: Doublecortin was originally discovered since defects in the gene encoding it are causative of X-linked lissencephaly, a rare group of brain malformations resulting in a smooth cerebral cortex caused by aberrant neuronal migration during development (1,2). The name Doublecortin comes from the unusual layering of the cortex in this form of lissencephaly, which appears to have a second deep cortical layer of neurons. This layer consists of neurons which did not migrate from the subventricular zone to the normal cortical layer. Patients with this defect suffer from seizures and mental retardation.

The HGNC name for Doublecortin is DCX, and it is also known as Doublin, Lissencephalin-X, DBCN and Lis-X. Four proteins encoded by the DCX produce bands of about 35 kDa and 45 kDa on Western blots (see the Uniprot page here). The 45 kDa form is known as Lis-XA while the smaller forms are generated by alternate transcription, are all missing the first 81 amino acids of Lis-XA, and are referred to as Lis-XB, Lis-XC, Lis-XD.

There are minor amino acid sequence differences between these three smaller isoforms. All of these protein contain two so-called Doublecortin domains, each about 90 amino acids long, which are believed to function in binding to microtubules, a C-terminal serine and proline rich region which may become phosphorylated *in vivo*. The doublecortin protein appears to function as a microtubule and actin binding protein and may interact with Lis-1, a member of the β-transducin or WD protein family, a protein mutations of which are also associated with lissencephaly. DCX is expressed very early in neuronal development, as neuroblasts become post-mitotic, but is lost as neurons mature. Developing neurons start to lose DCX expression about the time that they begin to express NeuN, a neuronal specific protein characteristic of more mature neurons, now known to correspond to the RNA binding protein Fox3.Antibodies to DCX are used to identify stem cells in sections and in tissue culture, and to see if neurogenesis is taking place. Our antibody stains identically to the Doublecortin (C18): sc-8066 polyclonal peptide antibody available from Santa Cruz.



Left: blots of crude rat brain extract from a postnatal 3 day animal stained with MCA-3E1. Two bands at ~45 kDa and ~35 kDa show that MCA-3E1 binds to an epitope in the region of DCX shared by Lis-A, and Lis-B, Lis-C and Lis-D, the C terminal 360 amino acids of Lis-A. **Right:** Rat brain neural cultures stained with MCA-3E1 (green), EnCor's chicken polyclonal antibody to MAP2 CPCA-MAP2 (red) and DNA (blue). The MCA-3E1 antibody reveals strong cytoplasmic staining in a population of small developing neurons. These cells are often found in small clumps as in these cultures, as in this particular example. Note that they are not positive for MAP2, which is characteristic of more mature neurons, and that the mature neuron shown is negative for MCA-3E1. This DCX antibody is therefore an excellent marker of developing neuronal cells.

Antibody characteristics: MCA-3E1 was raised in mouse against recombinant full length human Lis-A isoform of DCX purified from *E. coli*. The antibody is an IgG2a class antibody with a κ light chain. MCA-3E1 is known to react with Fox3/NeuN from human, cow, pig, mouse, rat and other mammals. Since Fox3/NeuN is highly conserved, it is likely that the antibody is effective on other species also.

Suggestions for use: The antibody solution is affinity purified from tissue culture supernatant and is at concentration of 1mg/ml in phosphate buffered saline. The antibody solution can be used at dilutions of at least 1:1,000 in immunofluorescence experiments. In western blotting using chemiluminescence it can be used at dilutions of 1:10,000 or lower. Antibody preparation contains 10 mM sodium azide preservative (Link to http://www.encorbio.com/MSDS/azide.htm for Material Safety Data Sheet). Avoid repeated freezing and thawing, store at 4°C or -20°C.

References:

1. des Portes V, Pinard JM, Billuart P, Vinet MC, Koulakoff A, Carrié A, Gelot A, Dupuis E, Motte J, Berwald-Netter Y, Catala M, Kahn A, Beldjord C and Chelly J. A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar heterotopia and lissencephaly syndrome. Cell 92:51-61 (1998).

2. Gleeson JG, Allen KM, Fox JW, Lamperti ED, Berkovic S, Scheffer I, Cooper EC, Dobyns WB, Minnerath SR, Ross ME and Walsh CA. Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. Cell 92:63-72 1998.

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