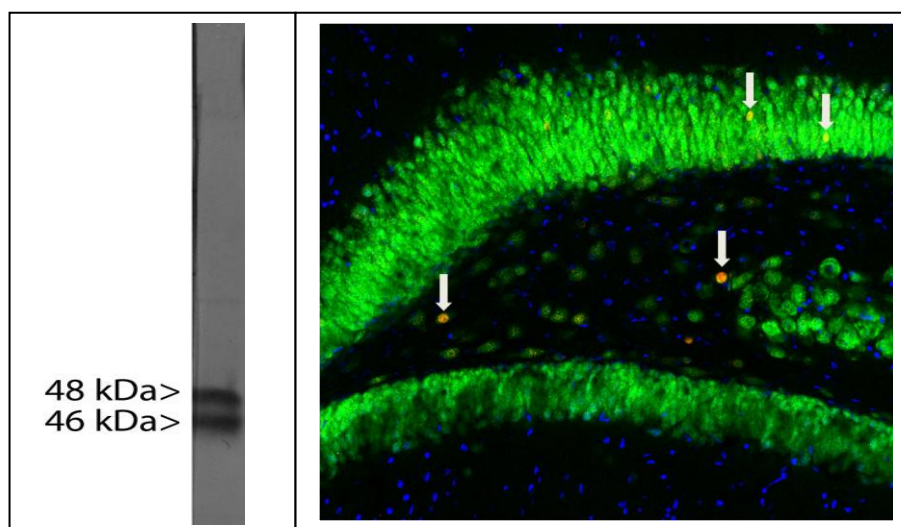


Catalogue# MCA-1B7: Monoclonal antibody to NeuN/Fox3: RBFOX3

The Immunogen: Fox3 is one of a family of mammalian homologues of Fox-1, which was originally discovered in *C. elegans* as a gene involved in sex determination. Fox is an acronym of "Feminizing locus on X". The mammalian genome contains three genes homologous to *C. elegans* Fox-1, called Fox1, Fox2 and Fox3. The Fox-1 protein and mammalian homologues are all about 46kDa in size, each of which includes a central highly conserved [RRM](#) type RNA recognition motif. This motif corresponds to a small ~70 amino acid structure consisting of 4 β -strands and two α -helices. An alternate name for Fox3 is hexaribonucleotide binding protein 3, and these proteins are believed to have a role in the regulation of mRNA splicing. Much interest has focused on Fox3 as a result of the recent finding that this protein corresponds to [NeuN](#), a neuronal nuclear antigen. NeuN was first described in 1994 by Mullen et al. (2), who raised a series of monoclonal antibodies to mouse antigens with the original intent of finding species specific markers. In the event they obtained a clone, called mAb A60, which proved to bind an antigen expressed in neuronal nuclei and to a lesser extent the cytoplasm of neuronal cells, and which appeared to work on all vertebrates. A few neuronal cell types were not recognized by the the NeuN antibody, such as cerebellar Purkinje cells, olfactory Mitral cells and retinal photoreceptors, but no non neuronal cells were recognized. The vast majority of neurons are strongly NeuN positive, and NeuN immunoreactivity has been widely used to measure the neuron/glial ratio in brain regions (3). The protein bound by this antibody was not characterized, though the molecular weight of this protein was shown to be closely spaced bands running at 46-48kDa on SDS-PAGE gels. The exact identity of the NeuN protein was not elucidated in this paper or, despite several attempts, until many years later. Despite this the mAb A66 antibody has become very widely used as a robust marker of neurons and neuronal stem cells, and a recent Medline search using the keyword "NeuN" produced over 1,100 hits. Recently Kim et al. used proteomic methods to show that NeuN corresponds to Fox3 (4). NeuN/Fox-3 is therefore a protein which has a function in RNA splicing and is expressed heavily and specifically in neuronal nuclei and cytoplasm. Our antibody was raised against the N-terminal 100 amino acids of human Fox3 as expressed in and purified from *E. coli*. We did not use full length Fox3 as immunogen since the three mammalian Fox homologues, namely Fox1, Fox2 and Fox3, include virtually identical [RRM](#) motifs. The N-terminal regions of the three molecules are much more variable so antibodies specific for each can therefore be generated. For a review of the Fox family of proteins see reference 5. The [HGNC](#) for this protein is [RBFOX3](#).



Left: blots of crude rat brain extract stained with MCA-1B7. Fox3/NeuN is expressed as two alternate transcripts with apparent SDS-PAGE molecular weight 46 and 48kDa. **Right:** Adult rat hippocampus stained with our rabbit antibody to c-Fos, [RPCA-c-Fos-AP](#) in red and DNA (blue). The MCA-1B7 antibody reveals strong nuclear and distal cytoplasmic staining for hippocampal neurons and the complete absence of staining of non-neuronal cells. This Fox3/NeuN antibody is therefore an excellent marker of neuronal cells. A few spontaneously

active cells show in addition expression of c-Fos (arrowed). This antibody is therefore an excellent marker of neuronal activation.

Antibody characteristics: MCA-1B7 is a mouse IgG2b class antibody. MCA-1B7 is known to react with Fox3/NeuN from human, cow, pig, mouse, rat and other mammals. It was raised against the N-terminal 100 amino acids of human Fox3, which were expressed in and purified from *E. coli*. The antibody does not cross react with either Fox1 or Fox2. 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 1:1,000 or lower. Antibody preparation contains 10mM 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.

Omim Link: Currently no OMIM entry.

References:

1. Hodgkin J, Zellan JD, Albertson DG. Identification of a candidate primary sex determination locus, fox-1, on the X chromosome of *Caenorhabditis elegans*. [Development 120:3681-3689 \(1994\)](#).
2. Mullen RJ, Buck CR, Smith AM. NeuN, a neuronal specific nuclear protein in vertebrates. [Development 116:201-211 \(1994\)](#).
3. Herculano-Houzel S, Lent R. Isotropic fractionator: a simple, rapid method for the quantification of total cell and neuron numbers in the brain. [J Neurosci. 25:2518-21 \(2005\)](#).
4. Kim KK, Adelstein RS, Kawamoto S. Identification of neuronal nuclei (NeuN) as Fox-3, a new member of the Fox-1 gene family of splicing factors. [J. Biol. Chem. 284:31052-31061 \(2009\)](#).
5. Underwood, J.G., Boutz, P.L., Dougherty, J.D., Stoilov, P. and Black, D.L. Homologues of the *Caenorhabditis elegans* Fox-1 protein are neuronal splicing regulators in mammals. [Mol. Cell. Biol. 25:10005-10016 \(2005\)](#).

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

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