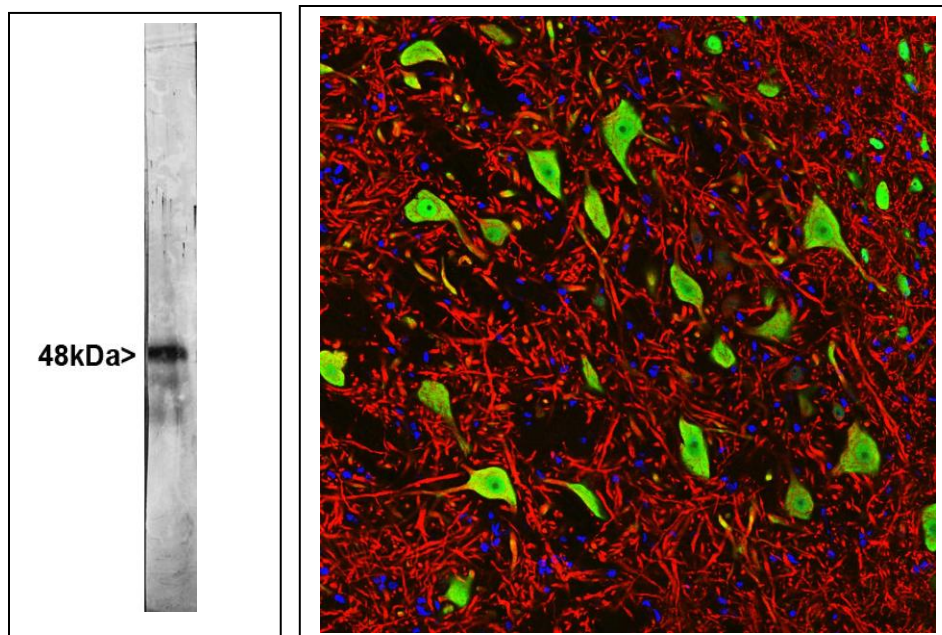


Catalogue RPCA-FOX3: Rabbit polyclonal antibody to FOX3/NeuN: RBFOX3

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 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/glia 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: Western blot of rat brain homogenates stained with RPCA-FOX3. Antibody binds closely spaced 48 and 46 kDa bands. **Right:** Paraformaldehyde fixed frozen section of adult rat brain stem stained with RPCA-Fox3 in green and counterstained for MAP2 with [MCA-5H11](#) in red. DNA is shown in blue. Note that RPCA-Fox3 stains

neuronal nuclei and distal perikarya and that the MAP2 antibody stains the dendrites extending from these cells. RPCA-Fox3 stains exactly like the original NeuN monoclonal antibody and like EnCor NeuN/Fox3 mouse monoclonal, [MCA-1B7](#). These antibodies do not bind to the nuclei of perikarya of any non-neuronal cells, so that they can be used to identify and quantify neurons.

Antibody Characteristics: The antibody was raised against a recombinant construct containing the first 100 amino acids of human Fox3. This construct was expressed in and purified from *E. coli*. The antibody is provided in the form of crude rabbit serum. Store at 4°C or -20°C. Avoid repeat freezing and thawing.

Suggestions for use: The serum can be diluted to 1:500-1,000 for immunofluorescence staining and 1:5,000-10,000 for western blotting. On western blots look for a major bands at 46 and 48 kDa.

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. 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 \(2009\)](#).
4. 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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