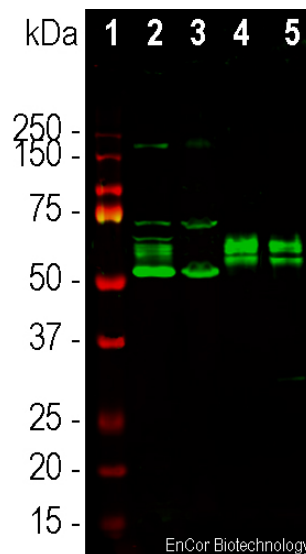


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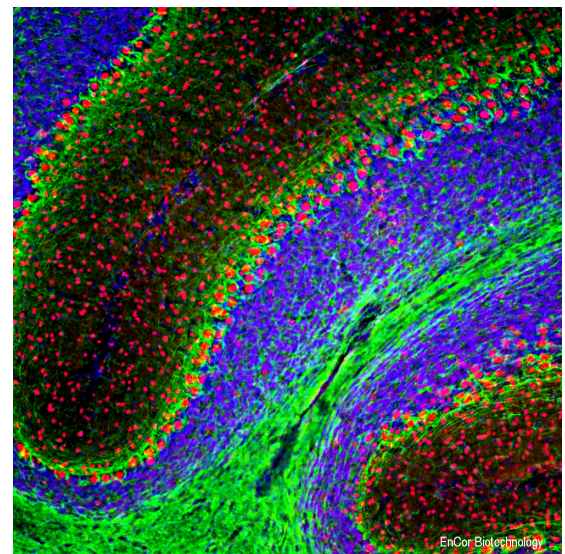
HGNC Name: RBFOX2
UniProt: O43251
RRID: AB_2744538
Immunogen: N-terminal 99 amino acids of human FOX2 expressed in and purified from *E. coli*
Format: Concentrated IgY preparation plus 0.02% Na₂S₂O₃
Storage: Store at 4°C.
Recommended dilutions:
 WB: 1:1,000. IF/IHC 1:2,000

References:
 1. Mullen RJ, Buck CR, Smith AM. NeuN, a neuronal specific nuclear protein in vertebrates. *Development* 116:201-11 (1994). 2. 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). 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.* 284:31052-61 (2009). 4. Underwood JG, et al. Homologues of the *Caenorhabditis elegans* Fox-1 protein are neuronal splicing regulators in mammals. *Mol. Cell Biol.* 25:10005-16 (2005). 5. Herculanou-Houzel S, Lent R. Isotropic fractionator: a simple, rapid method for the quantification of total cell and neuron numbers in the brain. *J. Neurosci.* 25:2518-2521 (2005). 6. Azevedo FA, et al. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J. Comp. Neurol.* 513:532-41 (2009). 7. Kuroyanagi H. Fox-1 family of RNA-binding proteins. *Cell Mol. Life Sci.* 66:3895-907 (2009).

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC	Chicken		50-68kDa	Hu, Rt, Ms



Western blot analysis of nuclear enriched fractions from different tissue and cells using chicken pAb to FOX2, CPCA-FOX2, in green: [1] protein standard, [2] rat brain, [3] mouse brain, [4] HEK293, and [5] NIH-3T3 cells. The bands between 50kDa and 75kDa correspond to the FOX2 protein.



Immunofluorescent analysis of mouse cerebellum section stained with chicken pAb to FOX2, CPCA-FOX2, dilution 1:2,000 in red, and costained with mouse mAb to NF-L, MCA-7D1, dilution 1:5,000 in green. The blue is Hoechst staining of nuclear DNA. Following transcardial perfusion of mouse with 4% paraformaldehyde, brain was post fixed for 24 hours, cut to 45 μm, and free-floating sections were stained with above antibodies. FOX2 antibody reveals protein expressed in the nuclei of certain granule cells and all Purkinje cells, while NF-L antibody labels dendrites and axons.

Background: In the early 90s an unusual protocol resulted in the raising of a mouse monoclonal antibody against a component of neuronal nuclei and proximal perikarya (1). The component was therefore named "NeuN" and was shown to correspond to two protein bands at 46 and 48kDa in SDS-PAGE blots. The antibody become very widely used as a reliable neuronal marker, apparently binding to neurons in all vertebrates. A few neuronal cell types were not recognized by the original NeuN antibody such as cerebellar Purkinje cells, olfactory mitral cells and many type of retinal neuron. However the vast majority of neurons are strongly NeuN positive, and NeuN immunoreactivity has therefore been widely used to identify neurons. The identity of the NeuN protein was however unknown until 2009 when Kim et al. (2) showed that it was identical to FOX3, a mammalian homolog of a gene product originally identified in *Caenorhabditis elegans* and named FOX1 (2). The *C. elegans* protein was discovered as it had a role in sex determination during early development, FOX being an acronym for "feminizing locus on the X chromosome" (3). There are three mammalian FOX1 protein homologs, namely FOX1, FOX2 and FOX3, which are all believed to have a role in the regulation of mRNA splicing (4). All three contain an almost identical central RNA recognition motif or RRM domain, a region of about 90 amino acids found in numerous proteins. Like FOX3/NeuN, FOX2 is expressed in neuronal nuclei, but interestingly in many neurons, such as Purkinje cells, which are FOX3/NeuN negative. Antibodies to FOX2 can therefore be used to add to the utility of FOX3/NeuN antibodies to identify and count neurons (5,6). FOX2 is also known as RBFOX2, RBM9, RTA and HRNBP2. For a review of the FOX1 family of RNA binding proteins see reference 7. The CPCA-FOX2 antibody was raised against a recombinant human FOX2 construct based only on the N-terminal sequence, not including the RRM domain and C-terminal regions. While the RRM region is identical between the three proteins, the N-terminal regions of FOX1, FOX2 and FOX3 are relatively poorly conserved so we were able to obtain antibodies which recognized FOX3 but not FOX2 or FOX1. As a result it is known that this antibody binds to the N-terminus of FOX2, specifically amino acids 1-100.

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Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry
 IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Co—Cow Pi—Pig Ho—Horse Ch—Chicken
 Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.