

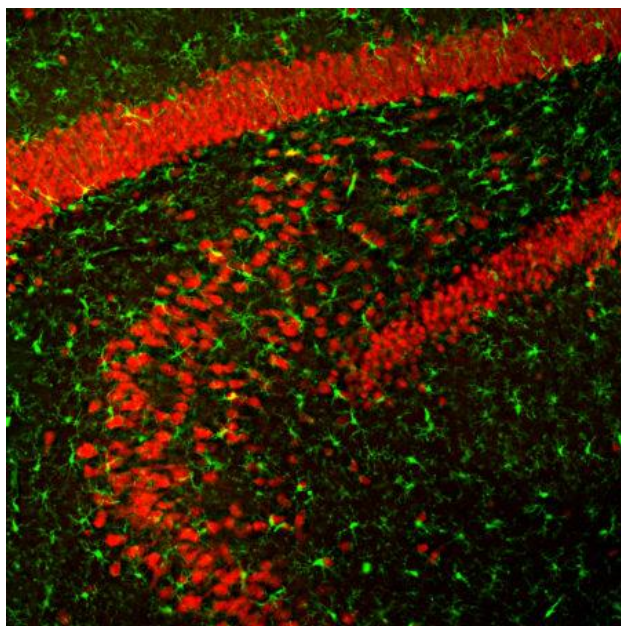


Catalog Number:	CH22131	Host:	Chicken
Product Type:	Chicken Polyclonal	Species Reactivity:	Human, Rat, Mouse
Immunogen Sequence:	N-terminal 100 amino acids of human FOX3 expressed in and purified from E. coli	Format:	Supplied as an aliquot of IgY preparation plus 5mM NaN3
Applications:	Immunofluorescence: 1:5,000-10,000 Immunohistochemistry: 1:5,000-10,000 Western Blot: 1:500-1,000		
Storage:	Dilutions listed as a recommendation. Optimal dilution should be determined by investigator. The antibody can be stored at 2° - 8° C for 12 months without detectable loss of activity. Avoid repeated freeze-thaw cycles.		

Application Notes

Description/Data

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. 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 became 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 discovered to be identical to FOX3, a mammalian homolog of a gene product originally identified in *Caenorhabditis elegans* and named FOX1. 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". There are three mammalian FOX1 protein homologs, FOX1, FOX2 and FOX3, which are believed to have a role in the regulation of mRNA splicing. All three contain an almost identical central RNA recognition motif or RRM domain, a region of about 90 amino acids found in numerous proteins. The differing protein isoforms of FOX3 result from alternate splicing of two exons which code for an insert close to the C-terminus and short C-terminal extension. The extension includes a C-terminal proline-tyrosine sequence preceded by a hydrophobic amino acids (Φ -PY) which is known to target proteins to the nucleus, apparently accounting for FOX3 being present in both the nuclei and the cytoplasm in certain neurons.

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This antibody was raised against a recombinant human FOX3 construct based only on the N-terminal sequence, not including the RRM domain and C-terminal regions. 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 the epitopes for CH22131 are known to be within this construct, specifically amino acids 1-99.

Image: Immunofluorescent analysis of rat hippocampus section stained with chicken pAb to FOX3/NeuN, CH22131, dilution 1:5,000 in red, and costained with rabbit pAb to IBA1, dilution 1:2,000, in green. Following transcardial perfusion with 4% paraformaldehyde, rat brain was post fixed for 24 hours, cut to 35µM, and free-floating sections were stained with the above antibodies. The FOX3/NeuN antibody stains the nuclei and distal perikarya of most neurons, while the IBA1 antibody specifically labels microglial cells.

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