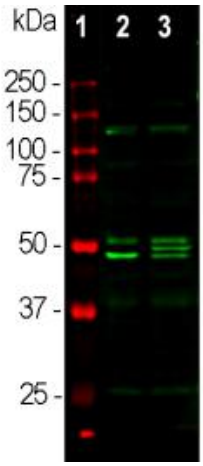
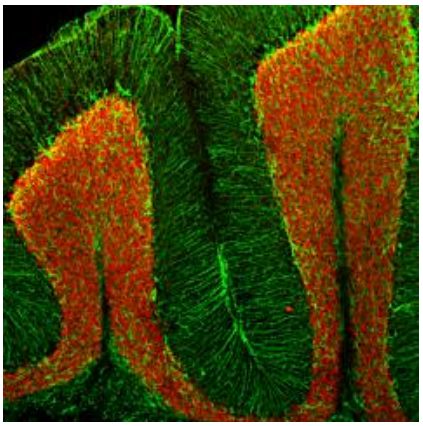
Product name:	NeuN-FOX3
Cat number:	AB-81163
Conjugate:	Unconjugated
Size:	100 ul
Clone:	POLY
Concentration:	1mg/ml
Host:	Rb
Isotype:	IgG
Immunogen:	N-terminal 100 amino acids of human FOX3 expressed in and purified from E. coli
Reactivity:	Hu, Ms, Rt
Applications:	WB: 1:5,000-1:10,000, ICC, IF: 1:500-1:1,000, IHC(F): 1:500-1:1,000
Molecular Weight:	46-48kDa
Purification:	Purified
Form:	Liquid
Buffer:	50% PBS, 50% glycerol plus 5mM NaN3
Storage:	4°C for short term and -20°C for longer term

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, FOX1, FOX2 and FOX3, which are 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. The differing protein isoforms of FOX3 result from alternate splicing of two exons which code for an insert close to the C-terminus and a short C-terminal extension (5). The extension includes a Cterminal proline-tyrosine sequence preceded by hydrophobic amino acids (Φ-PY) which is known to target proteins to the nucleus, apparently accounting for FOX3 being present in both nuclei and cytoplasm in certain neurons (5). The FOX3 antibody was raised against a recombinant human FOX3 construct based only on the N-terminal sequence, not including the RRM domain and Cterminal 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 FOX3 are known to be within this construct, specifically amino acids 1-99. We used the same recombinant immunogen to generate both chicken polyclonal and mouse monoclonal antibodies to FOX3, FOX3 and 1B7 respectively. These two antibodies also work in the same way as FOX3 and the original NeuN antibody and are versatile reagents which can be used in double and triple staining protocols. /NeuN immunoreactivity has been used to quantify the neuron/glial ratio in the brains of rats, humans and other species (6,7).



Western blot analysis of whole brain lysates using rabbit pAb to Fox3/NeuN,FOX3, dilution 1:1,000 in green: [1] protein standard (red), [2] mouse brain, [3] rat brain. Bands at 46-48kDa correspond to protein isotypes of the Fox3/NeuN protein.



Immunofluorescent analysis of a section of adult mouse cerebellum stained with rabbit pAb to FOX3/NeuN, FOX3, dilution 1:5,000 in red, costained with chicken pAb to GFAP, -GFAP, dilution 1:5,000, in green. Following transcardial perfusion with 4% paraformaldehyde, brain was post fixed for 24 hours and 45µm free-floating sections were stained with above antibodies. The FOX3/NeuN antibody stains the nuclei of neurons in the cerebellar granule layer. The GFAP antibody stains the processes of Bergmann glia in the molecular layer and astroglia in the granule and white matter layers.