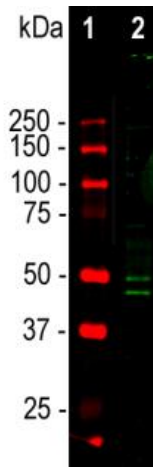
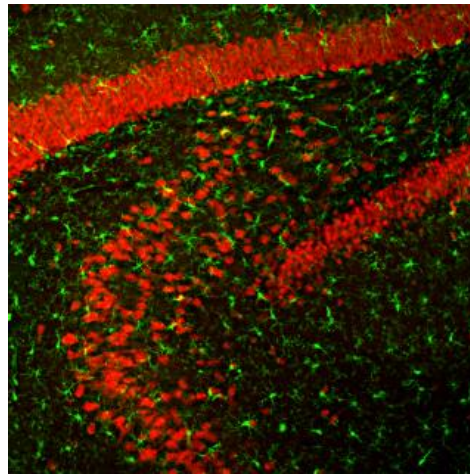

Product name:	NeuN-FOX3
Cat number:	AB- 83750
Conjugate:	Unconjugated
Size:	100 ul
Clone:	POLY
Concentration:	1mg/ml
Host:	Ch
Isotype:	IgY
Immunogen:	N-terminal 100 amino acids of human FOX3 expressed in and purified from E. coli.
Reactivity:	Hu, Ms, Rt
Applications:	WB: 1:500-1:1000, ICC: 1:5000-10000, IF: 1:5000-10000, IHC(F): 1:5000-10000
Molecular Weight:	46-48kDa
Purification:	Purified
Form:	Liquid
Buffer:	5mM NaN ₃ .
Storage:	Store at 4°C for short term, for longer term at -20°C

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 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 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 C-terminal 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 the nuclei and the cytoplasm in certain neurons (5). The Chicken FOX3 antibody was raised against a recombinant human FOX3 construct based only on the Nterminal 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 Chicken FOX3 are known to be within this construct, specifically amino acids 1-99.



Western blot analysis of mouse brain nuclear fraction lysate using chicken pAb to FOX3/NeuN, FOX3, dilution 1:1,000 in green. Lane 1 represents protein standard in red. Two bands at 46 and 50kDa mark correspond to the two isoforms of the FOX3/NeuN protein.



Immunofluorescent analysis of rat hippocampus section stained with chicken pAb to FOX3/NeuN, FOX3, dilution 1:5,000 in red, and costained with rabbit pAb to IBA1, Iba1, dilution 1:2,000, in green.

Following transcardial perfusion with 4% paraformaldehyde, rat brain was post fixed for 24 hours, cut to 35 μ m, 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.