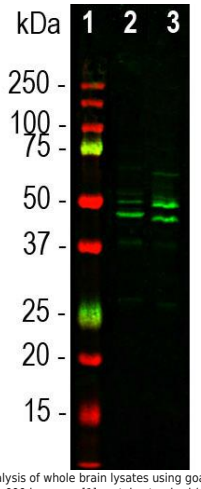
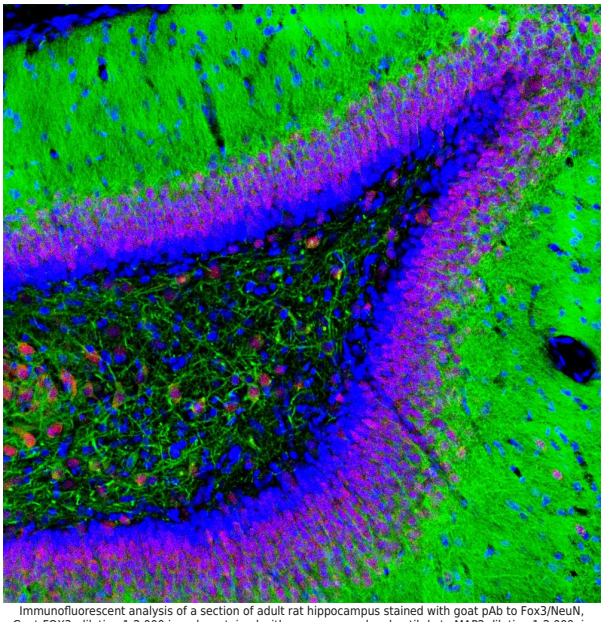
Product name:	NeuN-FOX3
Cat number:	AB-84292
Conjugate:	Unconjugated
Size:	100 ug
Clone:	POLY
Concentration:	1mg/ml
Host:	Gt
Isotype:	IgG
Immunogen:	N-terminal 100 amino acids of human Fox3 expressed in and purified from E. coli
Reactivity:	Hu, Ms, Rt
Applications:	Western blot: 1:1,000-1:2,000 Immunofluorescence: 1:1000-1:5,000 Immunohistochemistry:1:1000-1:5,000
Molecular Weight:	46-48kDa
Purification:	Purified
Form:	Liquid
Buffer:	Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM sodium azide
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 tested. 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 (2,3). 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 nuclei and cytoplasm in certain neurons (5). The Goat-FOX3 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 Goat-FOX3 are known to be within this construct, specifically amino acids 1-99. We used the same recombinant immunogen to generate chicken and rabbit polyclonals and mouse monoclonal antibodies to Neun/FOX3 respectively. These antibodies work in the same way as Goat-FOX3 and the original NeuN antibody and are versatile reagents which can be used in double and triple staining protocols. FOX3/NeuN immunoreactivity has been used to quantify the neuron/glial ratio in the brains of rats, humans and other species (6,7). Such studies can also be performed with any of our FOX3/NeuN reagents.



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



Immunofluorescent analysis of a section of adult rat hippocampus stained with goat pAb to Fox3/NeuN, Goat-FOX3, dilution 1:2,000 in red, costained with mouse monoclonal antibdy to MAP2 dilution 1:2,000, in green. Nuclear DNA was revealed in blue using the DAPI stain. Following transcardial perfusion of mouse with 4% araformaldehyde, brain was post fixed for 24 hours, cut to 45µM, and free-floating sections were stained with the above antibodies. The Fox3/NeuN antibody stains the nuclei of neurons in the ippocampus while the MAP2 antibody stains the dendritic processes of neurons.