Datasheet for 600-101-MJ0

Brn3b Antibody

Overview

Description:	Anti-Brn3b (GOAT) Antibody - 600-101-MJ0
Item No.:	600-101-MJ0
Size:	100 µg
Applications:	IF, IHC, WB
Reactivity:	Mouse, Rat
Host Species:	Goat

Product Details

Background:	Brn3b (Brain-Specific Homeobox/POU Domain Protein 3B) or POU4F2 (POU Class 4 Homeobox 2) is a DNA-binding transcriptional regulator and coregulator that recognizes and binds to the consensus octamer binding site 5-AT[A/T]A[T/A]T[A/T]A-3 in promoter of target genes. Brn3b plays a fundamental role in the gene regulatory network essential for retinal ganglion cell (RGC) differentiation. It cooperates with the transcription factor ISL1 to achieve RGC fate specification in the developing retina. And plays a role in RGC axon formation and guidance by regulating gene expression of specific target genes. Brn3b plays a role in TNFSF11-mediated terminal osteoclast differentiation. It binds chromatin at promoter region of target genes. It acts either as a transcriptional activator or repressor. It plays a role in the regulation of breast cancer cell growth by promoting transcription activation as well as repression of specific target genes. It is involved in tumor breast progression and invasion. Brn3b also plays a role either as a transcriptional coactivator or corepressor. Anti-Brn3b Antibody is useful for researchers interested in breast cancer research, transcription factor activity, and DNA binding.
Synonyms:	Goat Anti-Brn3b Antibody, Goat Anti-Pou4f2 Antibody, POU domain, class 4, transcription factor 2, Brain-specific homeobox/POU domain protein 3B, Brn-3B, Brain-3B, Brn-3.2
Host Species:	Goat
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	Pou4f2

Reactivity:	Mouse, Rat
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-Brn3b antibody was prepared from whole goat serum produced by repeated immunizations with a synthetic peptide corresponding to a near N-terminal portion of mouse Pou4f2 conjugated to Keyhole Limpet Hemocyanin (KLH).
Purity/Specificity:	This affinity purified antibody is directed against mouse Brn3b. This product was affinity purified from monospecific antiserum by immunoaffinity purification. A BLAST analysis was used to suggest cross-reactivity with the antigen based on 100% homology with the immunizing sequence to rat and 91.7% to human.
Relevant Links:	• UniProtKB - Q63934 • NCBI - NP-620394.2 • GeneID - 18997

Application Details

Tested Applications:	IF, IHC, WB
Application Note:	Anti-Brn3b Antibody has been tested in WB, IHC, and IF. Expect a band at ~43kDa in western blot using appropriate lysates. Positive controls used: Rat pup PND2-6 – minimal cortex brain lysate in WB, Mouse testis in IHC, Post-natal rat pup (PND1) heterogeneous brain cells in IF.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 50,000
IF:	5 µg/ml
IHC:	1:100
WB:	1:1000

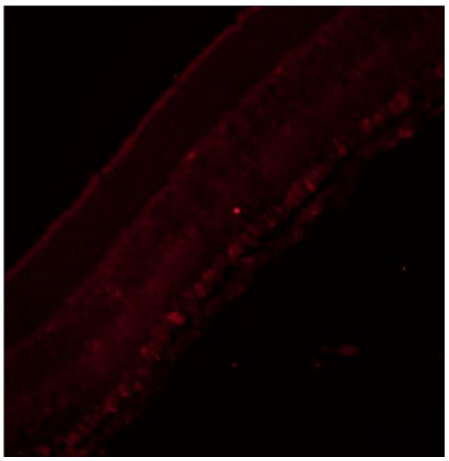
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.05 mg/ml by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

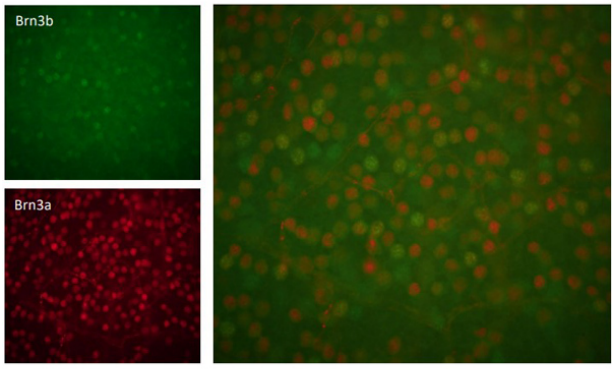
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

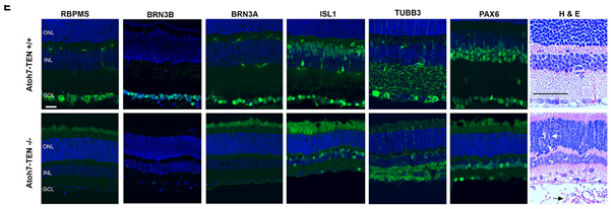
Images



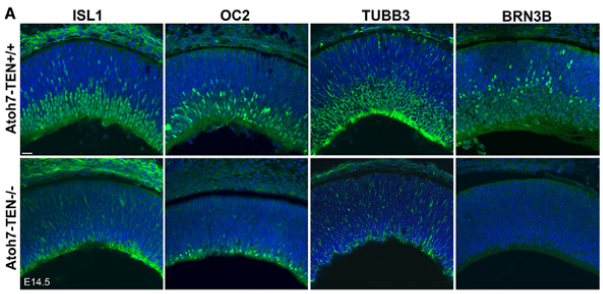
Immunofluorescence Microscopy
Immunofluorescence of Goat Anti-Brn3b Antibody. Tissue: Mouse Retina Tissue. Retrieval: 10mM Sodium Citrate pH6 retrieval and 10mM Tris-base 1mM EDTA 0.05% Tween 20 pH9. Permeabilization: 0.5% Triton X-100 per 15 mins. Primary Antibody: Anti-Brn3b at 1:200 overnight at 2-8°C. Incubate secondary antibody 2 hrs. Incubate HOECSHT 10 mins. Fluoro-G mounting. This antibody works with or without retrieval. Courtesy of Viviane Valenca at University of Utah.



Immunofluorescence Microscopy
Immunofluorescence of Goat Anti-Brn3b Antibody. Tissue: Mouse Retina Tissue. Permeabilization: 0.5%Triton X-100 per 45mins. Primary Antibody: Anti-Brn3b at 1:200 for 3 days at 2-8°C. Incubate secondary antibody for 2hrs. Incubate HOECSHT for 30 mins. Fluoro-G mounting. Courtesy of Viviane Valenca at University of Utah.



Immunofluorescence Microscopy
(E) Representative confocal images from adult WT and Atoh7-TEN−/− retinae stained for RBPMS, BRN3B, BRN3A, ISL1, TUBB3, and PAX6. Scale bar: 20 µm. H&E staining of WT and Atoh7-TEN−/− retinal sections from adult mice is also shown. Scale bar: 1 mm. Fig 2. PMID: 38823017



Immunofluorescence Microscopy
(A) Representative confocal images showing immunostaining of the RGC markers OC2, ISL1, and TUBB3 and BRN3B in WT and Atoh7-TEN−/− E14.5 retina. Scale bar: 20 µm. Fig 3. PMID: 38823017

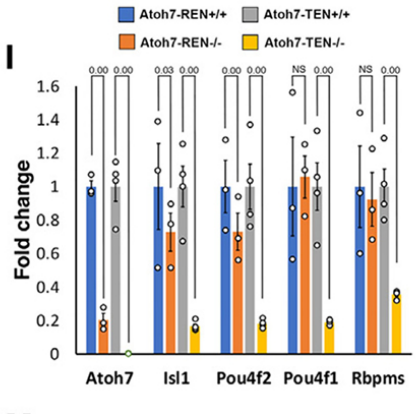
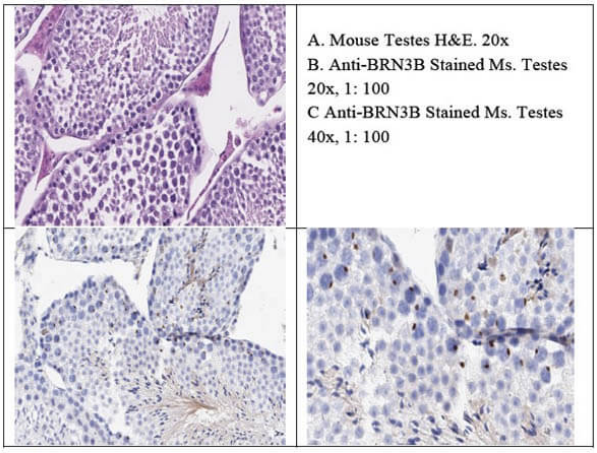
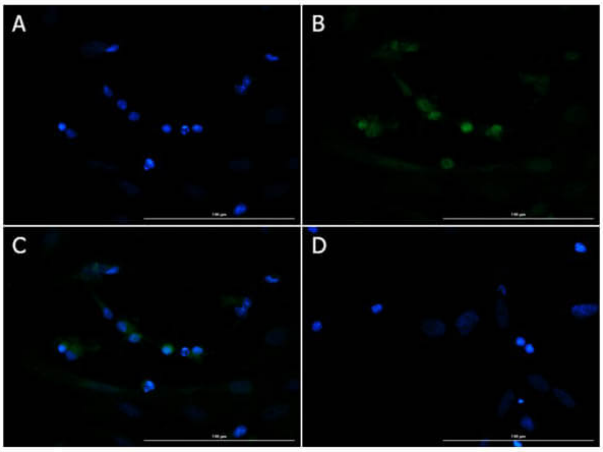


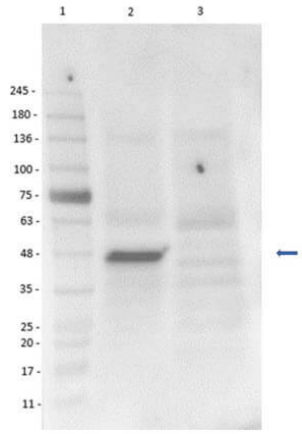
Figure
(I) Normalized RNA levels of Atoh7, Isl1, Pou4f2 (Brn3b), Pou4f1 (Brn3A), and Rbpms as revealed by RNA-seq of E14.5 retina in WT, ATOH7-REN-KO, and Atoh7-TEN-KO. Fig 4. PMID: 38823017



Immunohistochemistry
Immunohistochemistry of Goat Anti-Brn3b Antibody. Tissue: Mouse Testis. Fixative: none. Antigen Retrieval: HIER using Citrate Buffer for 20 minutes. Primary Antibody: Anti-Brn3b at 1:100 for 30 minutes at RT. Secondary Antibody: Donkey Anti-Goat IgG HRP 4µl/mL for 20 minutes at RT. Counterstain: Hematoxylin. Substrate: DAB. Analysis: BRN3B staining of human testes shows intense punctate intranuclear staining of Sertoli cell of the seminiferous tubules which is the expected staining pattern.



Immunofluorescence Microscopy
Immunofluorescence of Goat Anti-Brn3b Antibody. Cell Line: Post-natal rat pup (PND1) heterogeneous brain cells. Fixative: 4% PFA. Permeabilization: 0.3% Triton X-100. Primary Antibody: Anti-Brn3b at 5µg/mL overnight at 2-8°C. Secondary Antibody: Donkey Anti-Goat IgG DyLight™488 (p/n 605-741-125) at 15µg/mL for 1 hr at RT. Nuclear Counterstain: DAPI. Staining: (A). DAPI. (B). Brn3b + secondary. (C). Merge A+B. (D). secondary only. Expected localization: Nuclear, nuclear speckle.



Western Blot
Western Blot of Goat Anti-Brn3b Antibody. Lane 1: Opal Prestained Molecular weight ladder (p/n MB-210-0500). Lane 2: Rat pup PND2-6 – minimal cortex brain lysate (35µg) [+]. Lane 3: HEK293T whole cell lysate (35µg) [-]. Primary Antibody: Anti-Brn3b at 1:1000 overnight at 2-8°C. Secondary Antibody: Donkey anti-Goat IgG HRP (p/n 605-703-125) at 1:40,000 for 30 minutes at RT. Block: BlockOut Buffer (p/n MB-073). Exposure: 12 sec. Predicted MW: ~43kDa (rat), ~35, ~43kDa (mouse). Observed MW: ~47kDa.

References

- Mehta K et al. A cis-regulatory module underlies retinal ganglion cell genesis and axonogenesis. *Cell Rep.* (2024)

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