Human RBFOX3/NeuN Antibody

Monoclonal Mouse IgG_1 Clone # 1094645

Catalog Number: MAB11665

DESCRIPTION

Species Reactivity	Human
Specificity	Detects a synthetic peptide specific for human RBFOX3 around amino acid 5 in Direct ELISA.
Source	Monoclonal Mouse IgG_1 Clone # 1094645
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Synthetic Peptide Accession # A6NFN3
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose.

APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	2 µg/mL	Human hippocampus tissue
Multiplex Immunofluorescence	25 µg/mL	Immersion fixed paraffin-embedded sections of human cerebellum
Immunohistochemistry	3-25 µg/mL	Immersion fixed paraffin-embedded sections of human cerebellum

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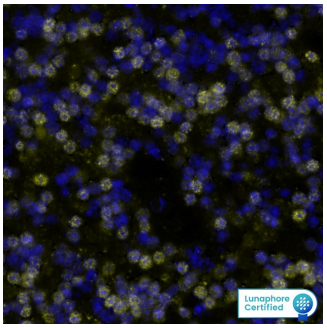
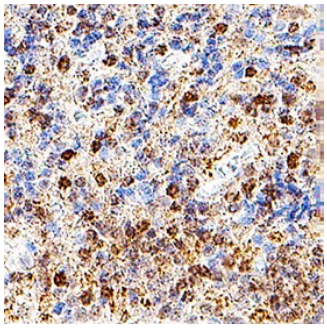
DATA

Multiplex Immunofluorescence



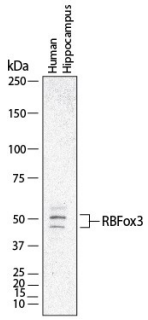
Detection of RBFOX3 in Human Cerebellum via seqIF™ staining on COMET™ RBFOX3 was detected in immersion fixed paraffin-embedded sections of human Cerebellum using Mouse Anti-Human RBFOX3, Monoclonal Antibody (Catalog #MAB11665) at 25ug/mL at 37 ° Celsius for 8 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; Epredia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ 647 Goat anti-Mouse IgG Secondary Antibody at 1:200 at 37 ° Celsius for 8 minutes. (Yellow; Lunaphore Catalog # DR647MS) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the neuronal nuclei. Protocol available in COMET™ Panel Builder.

Immunohistochemistry



Detection of RBFOX3/NeuN in Human Cerebellum. RBFOX3/NeuN was detected in immersion fixed paraffin-embedded sections of human cerebellum using Mouse Anti-Human RBFOX3/NeuN Monoclonal Antibody (Catalog # MAB11665) at 5 µg/ml for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001) or the HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the nucleus. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Western Blot



Detection of Human RBFOX3/NeuN by Western Blot. Western Blot shows lysates of human hippocampus tissue. PVDF membrane was probed with 2 µg/ml of Mouse Anti-Human RBFOX3/NeuN Monoclonal Antibody (Catalog # MAB11665) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). Specific bands were detected for RBFOX3/NeuN at approximately 40 and 50 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution	Reconstitute lyophilized material at 0.2mg/ml in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

RBFOX3, also known as Neuronal nuclei (NeuN), is a 34kDa marker of post-mitotic neurons that is highly conserved among different species. The RBFOX family regulates alternative slicing and is encoded by three genes: RBFOX1, RBFOX2 and RBFOX3. RBFOX3 mediates hippocampal circuitry, neurogenesis, and synaptogenesis. RBFOX3 plays a crucial role in normal synaptic function and is implicated in human neurological functions and mutations have been linked to neurodevelopmental delay, cognitive impairment, autistic features, and epilepsy.

References:

1. Wang HY, Hsieh PF, Huang DF, Chin PS, Chou CH, Tung CC, Chen SY, Lee LJ, Gau SS, Huang HS. RBFOX3/NeuN is Required for Hippocampal Circuit Balance and Function. Sci Rep. 2015 Dec 1;5:17383. doi: 10.1038/srep17383. PMID: 26619789; PMCID: PMC4664964.
2. Huang DF, Lee CY, Chou MY, Yang TY, Cao X, Hsiao YH, Wu RN, Lien CC, Huang YS, Huang HP, Gau SS, Huang HS. Neuronal splicing regulator RBFOX3 mediates seizures via regulating Vamp1 expression preferentially in NPY-expressing GABAergic neurons. Proc Natl Acad Sci U S A. 2022 Aug 16;119(33):e2203632119. doi: 10.1073/pnas.2203632119. Epub 2022 Aug 11. PMID: 35951651; PMCID: PMC9388145.

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