Product Datasheet

Bestrophin 1 Antibody (E6-6) - BSA Free
NB300-164

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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Reviews: 1 Publications: 49

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
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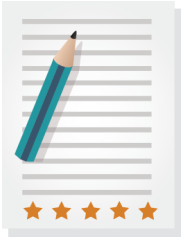
Updated 9/9/2025 v.20.1



NB300-164

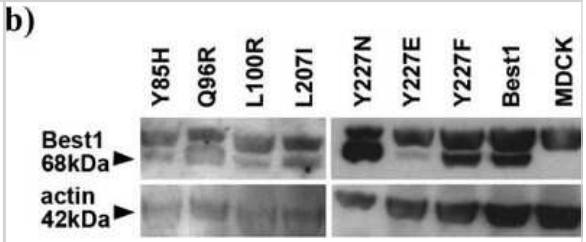
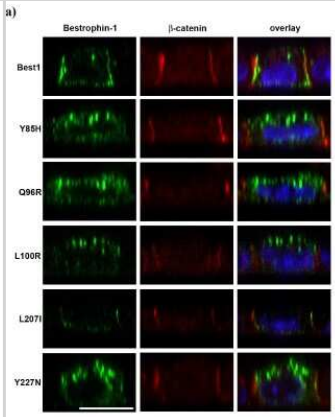
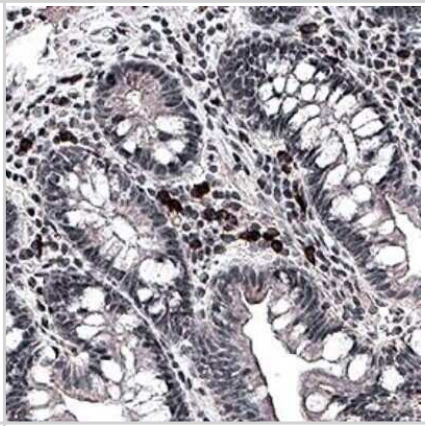
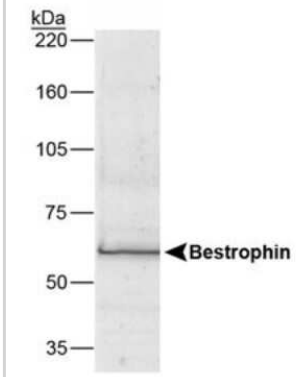
Bestrophin 1 Antibody (E6-6) - BSA Free

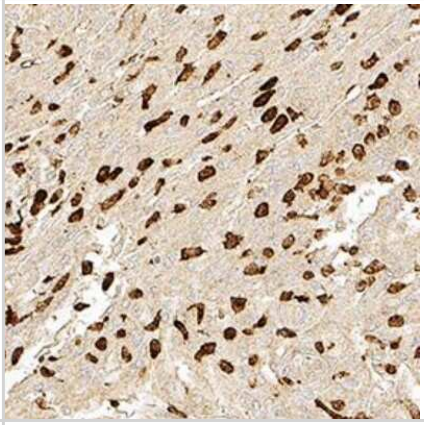
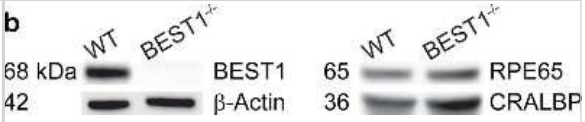
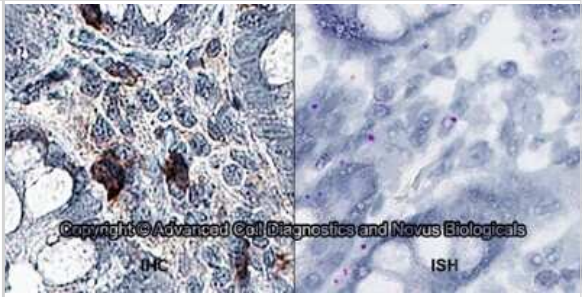
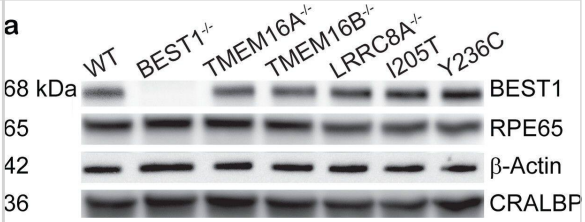
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	E6-6
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein A or G purified
Buffer	PBS

Product Description	
Description	Novus Biologicals Knockout (KO) Validated Mouse Bestrophin 1 Antibody (E6-6) - BSA Free (NB300-164) is a monoclonal antibody validated for use in IHC, WB, Dual RNAscope ISH-IHC, ICC/IF and IP. Anti-Bestrophin 1 Antibody: Cited in 49 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	7439
Gene Symbol	BEST1
Species	Human, Mouse, Porcine, Canine, Primate, Rat (Negative)
Reactivity Notes	Human, Primate, Porcine reactivity reported in scientific literature (PMID: 11050159). Use in Mouse reported in scientific literature (PMID:32791386).
Immunogen	Synthetic peptide conjugated to KLH corresponding to the C-terminus of human Bestrophin 1 (KDHMDPYWALENRDEAHS) [Uniprot: O76090]

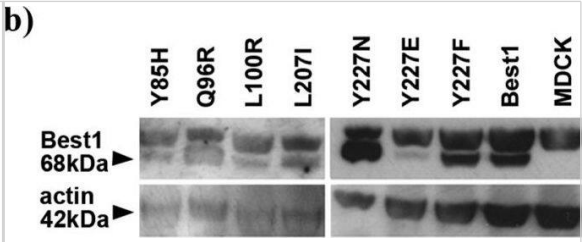
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunoprecipitation, Proximity Ligation Assay, Dual RNAscope ISH-IHC, Knockout Validated
Recommended Dilutions	Western Blot 1:1000, Immunohistochemistry reported in scientific literature (PMID 30048622), Immunocytochemistry/ Immunofluorescence, Immunoprecipitation, Immunohistochemistry-Paraffin reported in scientific literature (PMID 24345323), Immunohistochemistry-Frozen, Proximity Ligation Assay reported in scientific literature (PMID 27519691), Knockout Validated, Dual RNAscope ISH-IHC
Application Notes	In Western blot, this antibody recognizes a band at ~68 kDa representing Bestrophin. Please see protocol for treatment of cell extracts. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Images

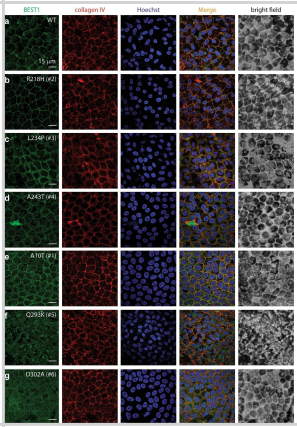
Western Blot: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - Western blot analysis of the normal and mutant human Best1 protein in transiently transfected MDCK cells. Best1 proteins are detectable as a 68 kDa band in all transfected cells, but not in non-transfected controls (MDCK lane). Actin bands are shown to indicate equal loading of cell lysates. Image collected and cropped by CiteAb from the following publication (https://www.mdpi.com/1422-0067/14/7/15121), licensed under a CC-BY license.	
Immunocytochemistry/Immunofluorescence: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - X-Z confocal single image scan of transiently transfected cells with different BEST1 cDNA constructs showing mislocalization of mutants Y85H, Q96R, L100R and Y227N. Cells were stained for Best1 (green), beta-catenin (red) and nuclei (blue). Scale bar = 10 um. Image collected and cropped by CiteAb from the following publication (https://www.mdpi.com/1422-0067/14/7/15121), licensed under a CC-BY license.	
Immunohistochemistry-Paraffin: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - Bestrophin 1 was detected in immersion fixed paraffin sections of human small intestine using t Mouse Anti-Human Bestrophin 1 Monoclonal Antibody (Catalog # NB300-164) at 5 ug/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the cell surface and extracellular.	
Western Blot: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - Detection of Bestrophin (68 kDa) from human RPE cell lysate.	

Immunohistochemistry-Paraffin: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - Bestrophin 1 was detected in immersion fixed paraffin-embedded sections of human brain using Mouse Anti-Human Bestrophin 1 (E6-6) Monoclonal Antibody (Catalog # NB300-164) at 1:300 for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the cytoplasm in neurons.	
Western Blot: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - Immunoblotting showing the expression of RPE-specific proteins BEST1 (NB300-164), RPE65 (NB100-355), CRALBP, and the loading control beta-Actin in iPSC-RPE cells. Two gels/blots in the same panel were prepared from the same cell lysate of each PSC-RPE to detect BEST1 + beta-Actin, and RPE65 + CRALBP, respectively. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/34061021/) licensed under a CC-BY license.	
Dual RNAscope ISH-IHC: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - Formalin-fixed paraffin-embedded tissue sections of human duodenum were probed for Bestrophin 1 mRNA (ACD RNAScope Probe, catalog # 433181; Fast Red chromogen, ACD catalog # 322360). Adjacent tissue section was processed for immunohistochemistry using mouse anti-human (Novus Biologicals catalog # NB300-164) at 0.3ug/mL with overnight incubation at 4 degrees Celsius followed by incubation with anti-mouse IgG VisUCyte HRP Polymer Antibody (Catalog # VC001) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). IHC signal is confined to cytoplasm.	
Expression of RPE-specific marker proteins in hPSC-RPE & iPSC-RPE cells.(a–b) Immunoblotting showing the expression of RPE-specific proteins BEST1, RPE65, CRALBP, & the loading control β-Actin in hPSC-RPE (a) & iPSC-RPE (b) cells. Two gels/blots in the same panel were prepared from the same cell lysate of each PSC-RPE to detect BEST1 + β-Actin, & RPE65 + CRALBP, respectively. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/34061021), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

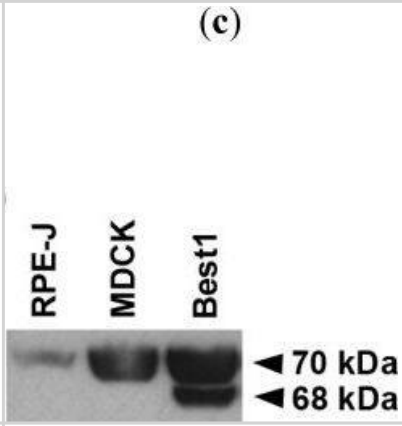
Western Blot: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - (a) Schematic drawing showing localization of the different mutations (black diamonds) tested in our study (modified from [12]); (b) Western blot analysis of the normal & mutant human Best1 protein in transiently transfected MDCK cells. Best1 proteins are detectable as a 68 kDa band in all transfected cells, but not in non-transfected controls (MDCK lane). Actin bands are shown to indicate equal loading of cell lysates. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23880862), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



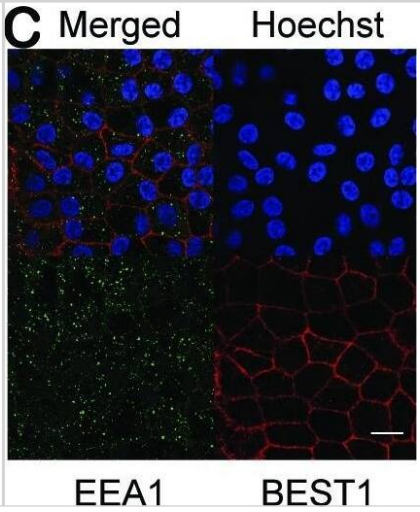
Immunocytochemistry/ Immunofluorescence: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - Subcellular localization of WT & mutant BEST1 in iPSC-RPEs. Confocal images showing the co-staining of BEST1, Collagen IV & Hoechst in iPSC-RPEs derived from a WT donor or patients. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31836750), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



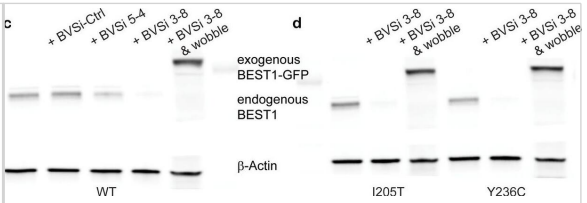
Western Blot: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - (a) HPRT & BEST1 mRNAs are expressed in MDCK & RPE-J cells. M–100 bp ladder, N–negative control; (b) Quantification of BEST1 expression levels between MDCK & RPE-J cells using quantitative Real-Time PCR. Fold change variation in BEST1 expression levels is reported as 2^-ΔΔCt value, the reference mRNA being HPRT (mean ± SEM., n = 2); (c) Western blot analysis—Best1 protein is not synthesized by RPE-J or MDCK cells. After transfection, MDCK produce human Best1 at 68 kDa. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23880862), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - Characterization of WT iPSC & iPSC-RPE. (A) Phase picture of established WT iPSC line before differentiation. Scale bar, 400 µm. (B) Immunocytofluorescence images of pluripotency markers in established iPSC. Scale bar, 200 µm. (C) Confocal images showing plasma membrane localization of BEST1. Scale bar, 10 µm. (D) Comparison of current amplitudes in iPSC-RPEs from two BEST1 WT donors. Bar chart showing the steady-state current amplitudes at 0 [Ca2+]i, 1.2 µM [Ca2+]i, & 1.2 µM [Ca2+]i + 100 µM NFA in RPEs from two distinct BEST1 WT human donors; n = 5–6. □$p<0.05 compared to current amplitudes at 1.2 µM [Ca2+]i from donor #1 & #2, respectively, using two-tailed unpaired Student t test. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29063836), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - CRISPR/Cas9-mediated gene silencing in combination with augmentation.(a) Augmented BEST1-GFP & endogenous BEST1 were detected by immunoblotting in hPSC-RPE cells. (b) Schematic of the baculovirus-based silencing (BVSi) vector. (c) Immunoblotting showing the knockdown of endogenous BEST1 expression with BVSi vectors & augmentation of wobble BEST1-mCherry in WT hPSC-RPE cells. (d) Immunoblotting showing the knockdown of endogenous BEST1 expression with BVSi 3–8 & augmentation of wobble BEST1-mCherry in hPSC-RPE cells carrying BEST1 gain-of-function mutations. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/34061021), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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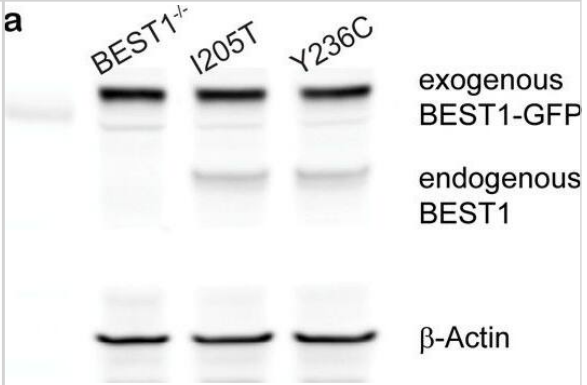
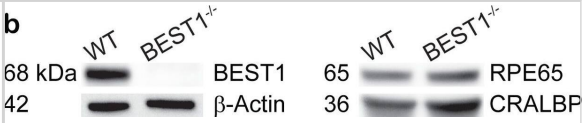
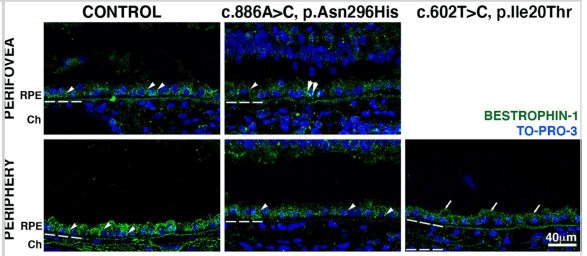
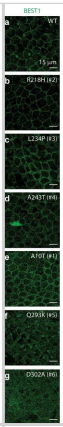
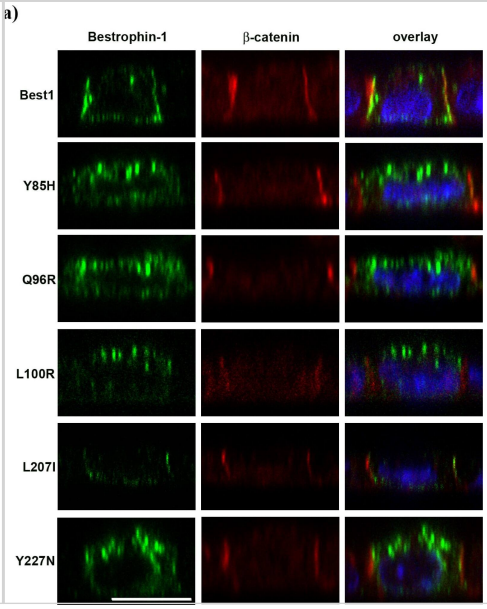


Western Blot: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - Expression of RPE-specific marker proteins in hPSC-RPE & iPSC-RPE cells.(a–b) Immunoblotting showing the expression of RPE-specific proteins BEST1, RPE65, CRALBP, & the loading control β-Actin in hPSC-RPE (a) & iPSC-RPE (b) cells. Two gels/blots in the same panel were prepared from the same cell lysate of each PSC-RPE to detect BEST1 + β-Actin, & RPE65 + CRALBP, respectively. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/34061021), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - Impact of BEST1 pathogenic variants in bestrophin-1 RPE localization. Cryosections obtained from the BD donors & an 88-year-old control were labeled with antibodies specific to bestrophin-1 (green), while cell nuclei have been labeled with TO-PRO-3 (blue). Bruch's membrane is indicated by the hashed white line. Arrow = mislocalized apical RPE distribution of bestrophin-1; arrowheads = basolateral RPE distribution of bestrophin-1; double arrowheads = intracellular bestrophin-1. Scale bar = 40 µm (all images). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/33154968), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - Subcellular localization of WT & mutant BEST1 in iPSC-RPEs. Confocal images showing the co-staining of BEST1, Collagen IV & Hoechst in iPSC-RPEs derived from a WT donor or patients. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31836750), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunocytochemistry/ Immunofluorescence: Bestrophin 1 Antibody (E6-6) - BSA Free [NB300-164] - (a) X-Z confocal single image scan of transiently transfected cells with different BEST1 cDNA constructs showing mislocalization of mutants Y85H, Q96R, L100R & Y227N. Cells were stained for Best1 (green), β-catenin (red) & nuclei (blue). Scale bar = 10 µm; (b) Z-series confocal stack signals corresponding to each labeling were quantified. Curves indicate the pixel intensity of each section along the Z-axis for each cell (Best1, green; β-catenin, red; nuclei, blue). The black vertical line indicates the Z-focal plane chosen as threshold for apical & basolateral domains separation. Basolateral & apical sides are as indicated. Horizontal axis represents µm distance & vertical axis shows pixel intensities; (c) Bar graph illustrating quantification of Best1 mutants distribution in the basolateral & apical domains of the cells compared with normal protein (mean ± SEM., n = 10, *p < 0.01, ***p < 0.0001). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23880862), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Li J, Qiu C, Wei Y et al. Human Amniotic Epithelial Stem Cell-Derived Retinal Pigment Epithelium Cells Repair Retinal Degeneration Frontiers in Cell and Developmental Biology 2021-09-28 [PMID: 34650985] (Immunoprecipitation, Western Blot, Immunohistochemistry-Frozen, Immunocytochemistry/ Immunofluorescence, Human, Porcine, Primate)

Afanasyeva TAV, Corral-Serrano JC, Garanto A et al. A look into retinal organoids: methods, analytical techniques, and applications Cellular and Molecular Life Sciences 2021-10-01 [PMID: 34420069] (Immunoprecipitation, Western Blot, Immunohistochemistry-Frozen, Immunocytochemistry/ Immunofluorescence, Human, Porcine, Primate)

Zhao Zhang, Bin Yan, Fei Gao, Qing Li, Xiaohong Meng, Peikai Chen, Lei Zhou, Wen Deng, Cheng Li, Weiyi Xu, Shuo Han, Hong Feng, Yaping Li, Junhui Chen, Zhengqin Yin, Can Liao, Hung-Fat Tse, Aimin Xu, Qizhou Lian PSCs Reveal PUFA-Provoked Mitochondrial Stress as a Central Node Potentiating RPE Degeneration in Bietti's Crystalline Dystrophy Molecular Therapy 2020-12-02 [PMID: 32755565]

Florian Udry, Sarah Decembrini, David M. Gamm, Nicole Déglon, Corinne Kostic, Yvan Arsenijevic Lentiviral mediated RPE65 gene transfer in healthy hiPSCs-derived retinal pigment epithelial cells markedly increased RPE65 mRNA , but modestly protein level Scientific Reports 2020-06-01 [PMID: 32483256]

Smith E, D'Antonio-Chronowska A, Greenwald W et al. Human iPSC-derived retinal pigment epithelium: a model system for identifying and functionally characterizing causal variants at AMD risk loci Stem Cell Reports 2019-05-14 [PMID: 31080113]

Bonilha, V L, Bell, B A Et al. Cellular Changes in Retinas From Patients With BEST1 Mutations. Front Cell Dev Biol 2020-11-07 [PMID: 33154968] (ICC/IF)

D'Antonio-Chronowska, A, D'Antonio, M Et al. In vitro Differentiation of Human iPSC-derived Retinal Pigment Epithelium Cells (iPSC-RPE). Bio Protoc 2019-12-20 [PMID: 33654959] (WB, Mouse)

Sripathi SR, Hu MW, Turaga RC Et al. Epithelial to mesenchymal transition (EMT) of human stem cell-derived retinal pigment epithelium shares commonalities with malignancy-associated EMT: a proteomic analysis Molecular & cellular proteomics : MCP 2021-08-26 [PMID: 34455105] (WB, Human)

Choudhury R, Bayatti N, Scharff R Et Al. FHL-1 interacts with human RPE cells through the alpha 5 beta 1 integrin and confers protection against oxidative stress Scientific reports 2021-07-08 [PMID: 34239032] (ICC/IF)

Schustak J, Twarog M, Wu X et al. Mechanism of Nucleic Acid Sensing in Retinal Pigment Epithelium (RPE): RIG-I Mediates Type I Interferon Response in Human RPE Journal of Immunology Research 2021-06-18 [PMID: 34239945] (ICC/IF, Human)

Zhao Q, Kong Y, Kittredge A et al. Distinct expression requirements and rescue strategies for BEST1 loss- and gain-of-function mutations eLife 2021-06-01 [PMID: 34061021] (WB)

Tsai YT, Li Y, Ryu J et al. Impaired cholesterol efflux in retinal pigment epithelium of individuals with juvenile macular degeneration American journal of human genetics 2021-04-21 [PMID: 33909993]

More publications at http://www.novusbio.com/NB300-164

Procedures

Western Blot protocol for Bestrophin 1 Antibody (NB300-164)

Procedure Guide for NB 300-164 Monoclonal Anti-Bestrophin

Western Blot Procedure

1. Run cell lysates** on an SDS-PAGE gel.
2. Transfer the proteins to PVDF.
3. Block the membrane in 1% Carnation instant milk in PBS + 0.1% Tween 20 (with 0.1mM CaCl2 and 1mM MgCl2) for 1 hour at RT.
4. Dilute the anti-Bestrophin [NB 300-164] to 1:1,000 in 10 ml of fresh blocking buffer and incubate for 1 hour at RT.
5. Wash the membrane with blocking buffer, 3x 5-10 minutes.
6. Dilute the secondary antibody in fresh blocking buffer, as recommended by the secondary vendor and incubate for 1hour at RT.
7. Wash the membrane with blocking buffer, 5x 8 minutes and rinse 1x with PBS (containing 0.1mM CaCl2 and 1mM MgCl2).
8. Detect the protein-antibody complex with alkaline phosphatase, if using NBT/BCIP or with HRP, if using ECL.

**Cell Lysate Preparation
A. Lysates were prepared in lysis buffer [50mM Tris-HCl, pH 8 / 120mM NaCl / 0.5% Nonidet P-40 / 10 ug/ml aprotinin / 10 ug/ml leupeptin / 1mM phenylmethylsulfonyl fluoride / 1mM sodium orthovanadate].
B. Total protein content was determined by bicinchoninic acid assay (Pierce).

Immunocytochemistry/Immunofluorescence Protocol for Bestrophin 1 Antibody (NB300-164)
Bestrophin 1 Antibody (E6-6):
Immunofluorescence

1. Paraffin slides. Deparaffinize as follows:
a. 2x 5 min in Xylene
b. 2x 5 min in 100% ethanol
c. 2x 5 min in 95% ethanol
d. 1x 5 min in 70% ethanol
e. 1x 5 min or more in PBS

2. Cryosections:
a. air dry for >30 min
b. rehydrate in PBS-CM (PBS + 0.1mM CaCl2 and 1mM MgCl2) + 3% BSA

3. Use pap pen to draw circles around sections
4. Block in PBS-CM + BSA for 30 min at RT
5. Dilute anti-Bestrophin [cat# NB 300-164] in PBS-CM + BSA and incubate at RT for 1 hour or overnight at 4C.
6. Wash the slides with PBS-CM + BSA 5x 5 min
7. Dilute the secondary antibody in PBS-CM + BSA and incubate at RT for >1 hour (if staining nuclei with propidium iodide add saponin to 0.1% and RNAse A at 1:500)
8. Wash 3x 8 min with PBS-CM + BSA and then 1x 5 min with PBS-CM
a. If staining nuclei with DAPI or propidium iodide, dilute into PBS-CM at 1:1000
b. Wash 3x with PBS-CM, if using propidium iodide
c. Proceed directly to step 9, if using DAPI
9. Mount in Flourmount.

**NOTE: Immunofluorescence Considerations
1. Aldehyde fixatives (ie: PFA and formalin) will not work in immunofluorescence with this antibody.
A) Transfected cells on coverslips can be fixed in acetone or methanol, as can tissue.
B) Paraformaldehyde for paraffin sections can be used if the tissue is subject to heat and pressure mediated antigen retrieval [see specific reference 1 on datasheet]
2. To date, endogenous protein in human or pig eyes cannot be detected, even in methanol/acetone fixed sections directly.
3. Immunohistochemistry, using this antibody, has been done using the vector ABC kit, which includes a signal amplification step.

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