Product Datasheet

RPE65 Antibody (401.8B11.3D9) - BSA Free
NB100-355

Unit Size: 0.2 ml

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

Reviews: 7 Publications: 93

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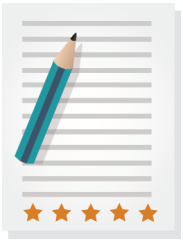
Updated 12/20/2023 v.20.1



NB100-355

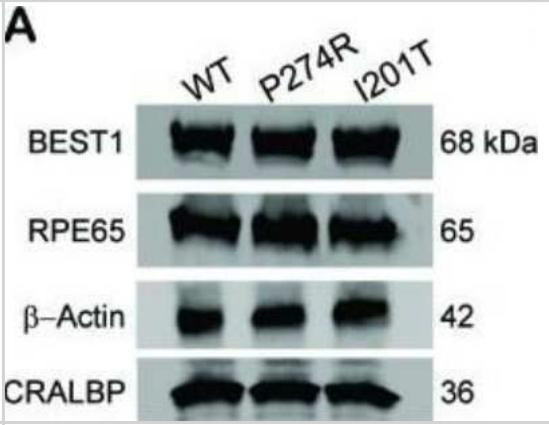
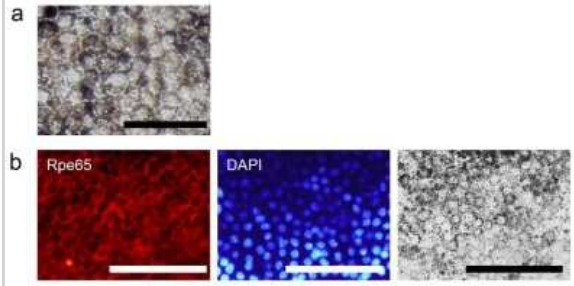
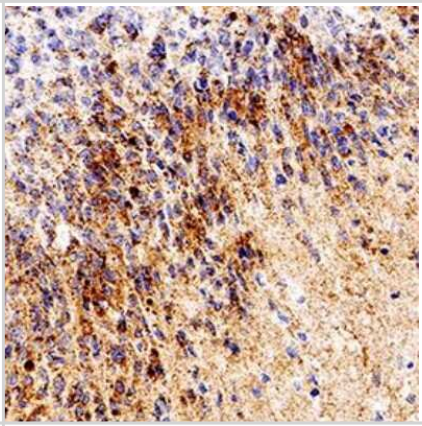
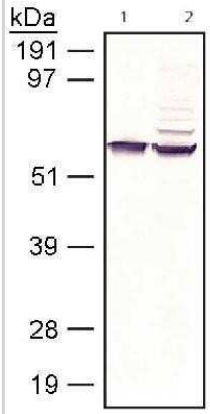
RPE65 Antibody (401.8B11.3D9) - BSA Free

Product Information	
Unit Size	0.2 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	401.8B11.3D9
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	65 kDa

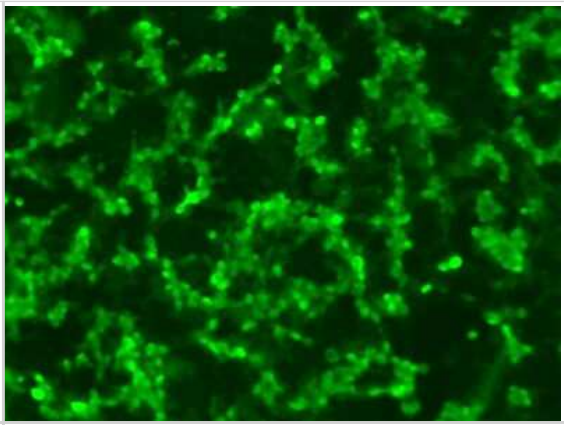
Product Description	
Host	Mouse
Gene ID	6121
Gene Symbol	RPE65
Species	Human, Mouse, Rat, Porcine, Bovine, Canine, Chicken, Primate, Xenopus
Reactivity Notes	Primate reactivity reported in scientific literature (PMID: 31660416).
Marker	Retinal Pigment Epithelium Marker
Immunogen	This RPE65 Antibody (401.8B11.3D9) was developed against bovine RPE65 microsomal membrane proteins. [UniProt# Q28175]

Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vivo assay, Immunoprecipitation, CyTOF-ready, Immunofluorescence, Knockout Validated
Recommended Dilutions	Western Blot 1 - 2 ug/ml, Simple Western, Flow Cytometry, Immunohistochemistry 1:250, Immunocytochemistry/ Immunofluorescence 1:50 - 1:200, Immunoprecipitation reported by customer review, Immunohistochemistry-Paraffin 1:250-1:500, Immunohistochemistry-Frozen 1:250, In vivo assay reported in scientific literature (PMID 32173468), Immunofluorescence 1:50 - 1:200, CyTOF-ready, Knockout Validated
Application Notes	For Western blot, this antibody has been validated in lysates of bovine RPE membrane and COS7 cells transfected with human RPE65, and in both samples, the antibody recognized a band at ~65 kDa, representing RPE65 protein. Note:- Hamel et al. have reported that in cultured bovine RPE cells, the levels of RPE65 are undetectable in WB after day 14, however, the mRNA levels are detectable by Northern for at least 7 weeks (PMID: 8340400). 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. This antibody is CyTOF ready. Simple Western reported by an internal validation. Separated by Size

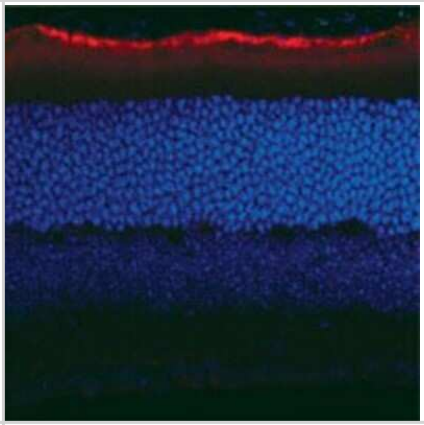
Images

Western Blot: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - Subcellular localization of BEST1 and surface Ca2+-dependent Cl- current in patient-derived iPSC-RPEs. Western blots show similar BEST1 expression levels in WT and patient-derived iPSC-RPEs. Each sample was from one cell lysis (BEST1 and beta-actin, RPE65 and CRALBP were on two gels, respectively). Image collected and cropped by CiteAb from the following publication (https://elifesciences.org/articles/29914), licensed under a CC-BY license.	
Immunocytochemistry/Immunofluorescence: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - Expression of eye-specific markers in the induced eye-like structures induced from lignin-added ES cells. (a) Higher-magnification image of the RPE like structure induced from ESCs after 12-day culture. (b) Immunostaining of eye- like structures. Eye- like structures induced from ESCs after 12-day culture were stained with antibodies against RPE65 (red) and nuclei were stained with DAPI solution (blue). Scale bar: a = 50 um, b,d = 200 um, c,e = 100 um. PLoS One. 2013 Jun 21;8(6):e66376. doi: 10.1371/journal.pone.0066376.	
Immunohistochemistry-Paraffin: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - Analysis of FFPE human glioblastoma tissue section using 1:500 dilution of RPE65 [401.8B11.3D9] antibody on a Bond Rx autostainer (Leica Biosystems). The assay involved 20 minutes of heat induced antigen retrieval (HIER) with 10mM sodium citrate buffer (pH 6.0) and endogenous peroxidase quenching using peroxide block. The sections were incubated with primary antibody for 30 minutes. Bond Polymer Refine Detection (Leica Biosystems) and DAB were used for signal detection which followed counterstaining with hematoxylin. Whole slide scanning and capturing of representative images (20X) were performed using Aperio AT2 (Leica Biosystems).	
Western Blot: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - WB analysis of RPE65 in 20ug lysate of COS7 cells expressing recombinant Human RPE65 (Lane 1) and 5ug of Bovine retinal pigment epithelium membrane fraction (Lane 2). Blot processed for detection with alkaline phosphatase conjugated goat-anti Mouse IgG secondary antibody and NBT/BCIP substrate.	

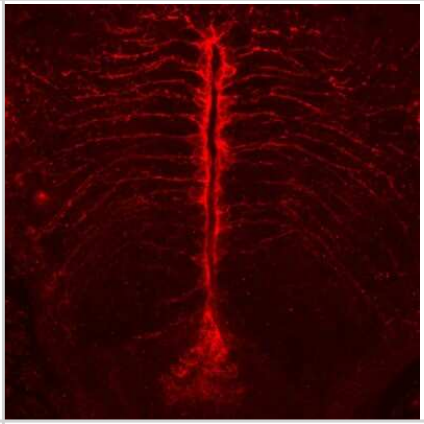
Immunocytochemistry/Immunofluorescence: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - ICC-IF analysis of cultured ARPE19 cells, a spontaneously arising human retinal pigment epithelia cell line - 10 minutes fixation in 4% PFA, 10 minutes permeabilization in PBS containing 0.2% Triton X-100 (PBS-T), 1 hour blocking in 10% normal goat serum containing 1% BSA in PBS-T, 1:100 primary antibody dilution in PBS, ON 4C incubation.



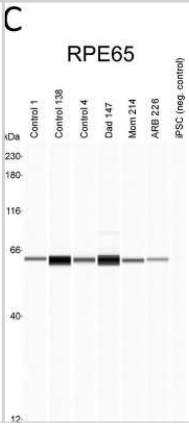
Immunohistochemistry-Frozen: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - Staining of RPE65 in a cryosection of mouse retina tissue using RPE65 antibody (clone 401.8B11.3D9).



Immunohistochemistry: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - Zebrafish brain ventricular area labeled with mouse anti-RPE65 (1:100). This image was submitted through a verified customer review.

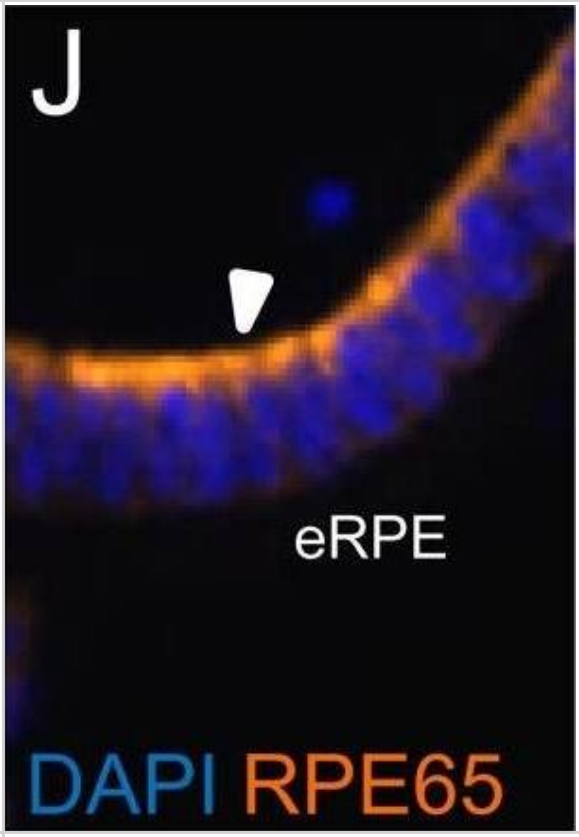


Simple Western: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - iPSC-RPE from all donors express RPE marker proteins. iPSC-RPE from the patient, the patients mom, the patients dad, and the three unrelated, unaffected controls all expressed RPE65. The negative control (iPSCs) had no detectable RPE65 protein expression. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/29540715/) licensed under a CC-BY license.

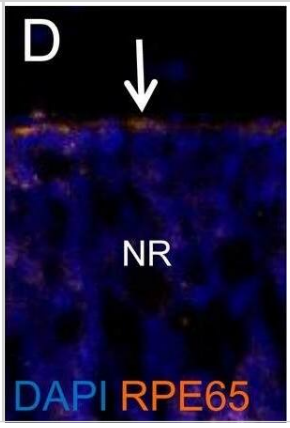


Knockout Validated: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - Immunoblotting showing the expression of RPE-specific proteins BEST1 (NB300-164), RPE65 (NB100-355), CRALBP, and the loading control beta-Actin in iPSC-RPE cells. Two gels/blots in the same panel were prepared from the same cell 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.

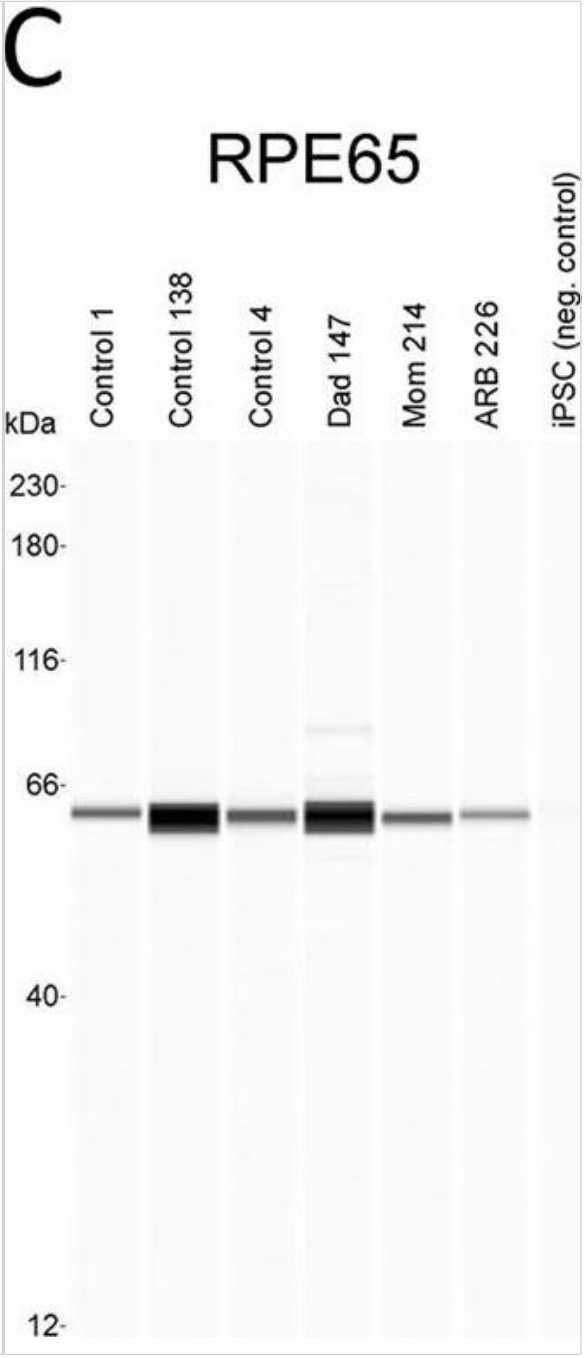
Attenuation of mTORC1/2 kinase ablates CPT-induced p53- and p53-dependent responses in ccRCC. (a) The 786-O and 786-O/VHL cells were treated with 400 nM pp242, 2 μM CPT, 10 μM nutlin-3a (N3) or vehicle control (DMSO) alone for 24 h, or preincubated with 400 nM pp242 for 1 h before addition of 2 μM CPT or 10 μM N3 for a further 24 h. Whole-cell lysates were assayed by western blot for HIF-2α, mTOR, phosphorylated p53 (S15), mTOR (S2448) and p70S6K (T389) proteins. Actin was used as a loading control. Short and long exposure times are shown for P-S15-p53. (b) RCC4 and RCC4/VHL cells were treated with 400 nM pp242 or vehicle control (DMSO) alone or preincubated with 400 nM pp242 for 1 h before addition of either 1 μM or 2 μM CPT for 24 h. Whole-cell lysates were assayed by western blot for mTOR, p53, phosphorylated p53 (S15), mTOR (S2448) and p70S6K (T389) proteins. Actin was used as a loading control. (c) RCC4 and RCC4/VHL cells were preincubated with 400 nM pp242 for 1 h before addition of 2 μM CPT for 24 h and mRNA expression of ET-1 and PAI-1 were assessed by real-time quantitative PCR relative to GAPDH. Mean±S.E. of duplicate values of one representative experiment is shown. (d) RCC4 cells were pretreated with 400 nM pp242 for 1 h before addition of 2 μM CPT for 24 h. Conditioned media were harvested and assessed for secreted ET-1 protein levels by ELISA and normalized to total protein levels. Mean ±S.E. of duplicate values of one representative experiment is shown Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/24136229), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Depletion of TCAB1 reduced the cell proliferation in vitro. a. qPCR results. Using exogenous shTCAB1 lentivirus (4 different shRNA targets) depleted TCAB1 mRNA significantly. b. Protein level also significantly decreased after shTCAB1 lentivirus treatment. c. Depletion of TCAB1 by exogenous shRNA decreased cell proliferation in Cal-27, ACC2 and HSC-3 cells. d. FACS results. Depletion of TCAB1 might facilitate cancer cell arrest, and shTCAB1-treated cells are significantly less in S phase. Statistical analysis was determined by Student's t test (**P < 0.01, ***P < 0.005). Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/25070141), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Cortisol inhibited selective autophagy towards SCG10 via activation of mTOR.a The cells were treated with cortisol (1 μM) for 3 h, and stained with rhod-2 (3 μM) for 1 h to detect mitochondrial Ca2+. After incubation, mitotracker green (MTG, 300 nM) was also stained to visualize mitochondria. The intensity of both rhod-2 (red) and MTG (green) was measured with Eclipse Ts2™ fluorescence microscopy. ** indicates $p < 0.01$ vs. control. Scale bars represent 100 μm (magnification, ×400). n = 5. b The cells were treated with RU 486 (1 μM) for 30 min before cortisol (1 μM) for 6 h, and then reacted with ATP luciferase reagent. The ATP levels were detected with luminometer. ** indicates $p < 0.01$ vs. control and ## indicates $p < 0.01$ vs. cortisol alone. n = 6. c Time responses (0–6 h) of cortisol (1 μM) in phosphorylation of AMPK at Thr172 and mTOR at Ser2448 were shown. ** indicates $p < 0.01$ vs. control. n = 4. d The cells were treated with xestospongin C (1 μM) for 2 h or ruthenium red (100 nM) for 30 min before cortisol (1 μM) for 6 h. p-mTOR (Ser2448), mTOR, and β-actin were detected in western blotting results. * indicates $p < 0.05$ vs. control and # indicates $p < 0.05$ vs. cortisol alone. n = 4. e The cells were pretreated with rapamycin (100 nM) for 30 min before cortisol (1 μM) for 24 h. Atg5, p62, LC3, and β-actin were detected with western blot. ** indicates $p < 0.01$ vs. control. #, ## indicates $p < 0.05$, $p < 0.01$ vs. cortisol, respectively. n = 5. f Time responses (0–24 h) of cortisol (1 μM) in stathmin-1 and SCG10 expressions were shown. *, ** indicates $p < 0.05$, $p < 0.01$ vs. control, respectively. n = 5. g The cells were pretreated with rapamycin (100 nM) for 30 min before cortisol (1 μM) for 24 h. SCG10 and β-actin were detected with western blot. * indicates $p < 0.05$ vs. control and ## indicates $p < 0.01$ vs. cortisol. n = 4. h The cells were pretreated with rapamycin (100 nM) for 30 min before cortisol (1 μM) for 24 h. SCG10 was co-immunoprecipitated with an anti-ubiquitin antibody (the left side). Expression of ubiquitin, SCG10, and β-actin in total cell lysates is shown in the right side. ** indicates $p < 0.01$ vs. control and ## indicates $p < 0.01$ vs. cortisol alone. n = 4. i The cells were pretreated with rapamycin (100 nM) for 30 min before cortisol (1 μM) for 24 h. Co-localization of LC3 (green) and SCG10 (red) was visualized with SRRF imaging system. DAPI was used for nuclear counterstaining (blue). ** indicates $p < 0.01$ vs. control and ## indicates $p < 0.01$ vs. cortisol alone. Scale bars represent 20 μm (magnification, ×1,000). n = 5. All blot and immunofluorescence images are representative. Quantative data are presented as a mean ± S.E.M. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/30429451), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Souverein EA, Nagiel A, Gnedeva K Obtaining High-Quality Cryosections of Whole Rabbit Eye Journal of visualized experiments : JoVE 2023-11-10 [PMID: 38009731]

Hernandez BJ, Skiba NP, Plößl K et al. Polarized Desmosome and Hemidesmosome Shedding via Exosomes is an Early Indicator of Outer Blood-Retina Barrier Dysfunction bioRxiv : the preprint server for biology 2023-06-13 [PMID: 37398366] (WB)

Details:
Dilution: 1:1000

Ogura S, Baldeosingh R, Bhutto IA et al. A role for mast cells in geographic atrophy The FASEB Journal 2020-08-01 [PMID: 32525594] (IHC)

Dhingra A, Tobias JW, Boesze-Battaglia K Transcriptomic changes predict metabolic alterations in LC3 associated phagocytosis in aged mice bioRxiv : the preprint server for biology 2023-03-14 [PMID: 36993501]

González-Zamora J, Hernandez M, Recalde S et al. Matrix Metalloproteinase 13 Is Associated with Age-Related Choroidal Neovascularization Antioxidants (Basel, Switzerland) 2023-04-05 [PMID: 37107259] (ICC/IF, Human)

Blomfield AK, Maurya M, Bora K et al. Ectopic Rod Photoreceptor Development in Mice with Genetic Deficiency of WNT2B Cells 2023-03-28 [PMID: 37048106] (IHC-Fr, Mouse)

Wu J, Cho CS, Jo DH, Kim JH Application of Base Editor-Mediated Genome Editing in Mouse Retina Methods in molecular biology (Clifton, N.J.) 2023-01-02 [PMID: 36592316]

Jo DH, Jang HK, Cho CS et al. Visual function restoration in a mouse model of Leber congenital amaurosis via therapeutic base editing Molecular therapy. Nucleic acids 2023-03-14 [PMID: 36589710] (IHC-P, Mouse)

Details:
Dilution used in IHC-P 1:100

Ripolles-Garcia A, Chen Y, Sato Y et al. Retinal Vascular Plexuses Are Unequally Affected in Canine Inherited Retinal Degenerations Investigative ophthalmology & visual science 2022-11-01 [PMID: 36378130] (IHC-Fr, Canine)

Details:
Dilution used in IHC-Fr 1:500

SimOn MV, Vera MS, Tenconi PE et al. Sphingosine-1-phosphate and ceramide-1-phosphate promote migration, pro-inflammatory and pro-fibrotic responses in retinal pigment epithelium cells Experimental eye research 2022-08-27 [PMID: 36041511] (ICC/IF, Human)

Tian H, Chen Z, Zhu X et al. Induced retinal pigment epithelial cells with anti-epithelial-to-mesenchymal transition ability delay retinal degeneration iScience 2022-09-01 [PMID: 36185374] (WB, IHC-Fr, Human)

Details:
WB dilution used 1:1000

Nguyen Hoang AT, Lee H, Lee SJ Casein kinase I inhibitor D4476 influences autophagy and apoptosis in chloroquine-induced adult retinal pigment epithelial-19 cells Experimental eye research 2022-02-24 [PMID: 35219693] (IF/IHC, Mouse)

More publications at http://www.novusbio.com/NB100-355

Procedures

Western Blot protocol for RPE65 Antibody (NB100-355)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

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Products Related to NB100-355

NBP2-46608	Mouse Eye Tissue Lysate (Normal)
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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