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.

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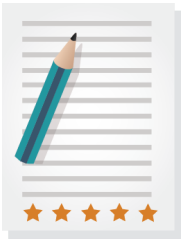
Updated 4/13/2025 v.20.1



NB100-355

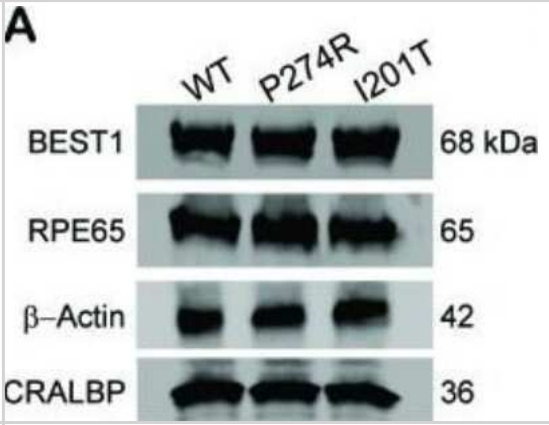
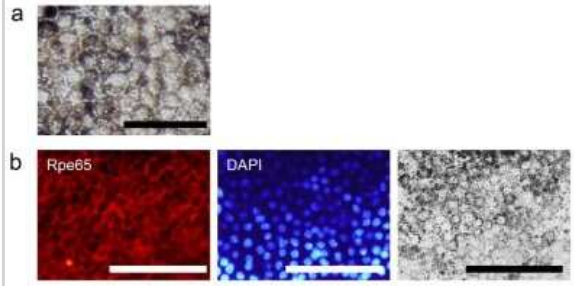
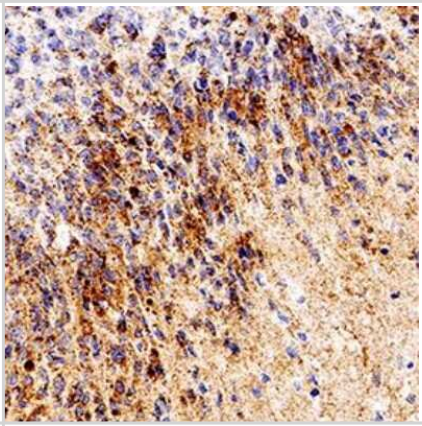
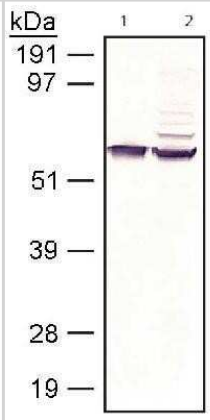
RPE65 Antibody (401.8B11.3D9) - BSA Free

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

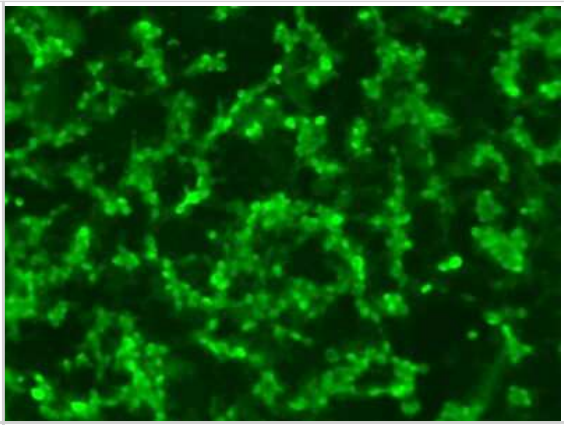
Product Description	
Host	Mouse
Gene ID	6121
Gene Symbol	RPE65
Species	Human, Mouse, Rat, Porcine, Bovine, Canine, Chicken, Primate, Xenopus
Reactivity Notes	Primate reactivity reported in scientific literature (PMID: 31660416).
Marker	Retinal Pigment Epithelium Marker
Immunogen	This RPE65 Antibody (401.8B11.3D9) 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. See Simple Western Antibody Database for Simple Western validation: tested in iPSC-RPE; separated by size

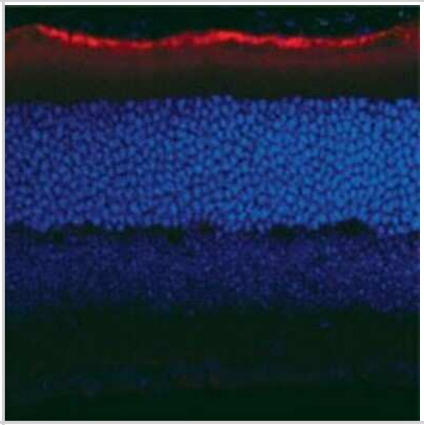
Images

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.	

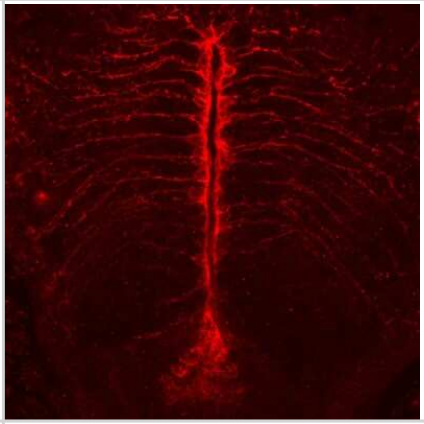
Immunocytochemistry/Immunofluorescence: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - ICC-IF analysis of cultured ARPE19 cells, a spontaneously arising human retinal pigment epithelia cell line - 10 minutes fixation in 4% PFA, 10 minutes permeabilization in PBS containing 0.2% Triton X-100 (PBS-T),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



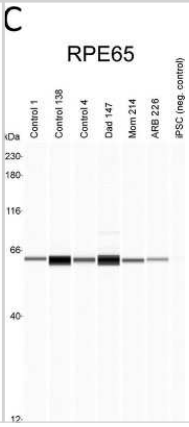
Immunohistochemistry-Frozen: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - Staining of RPE65 in a cryosection of mouse retina tissue using RPE65 antibody (clone 401.8B11.3D9).



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.

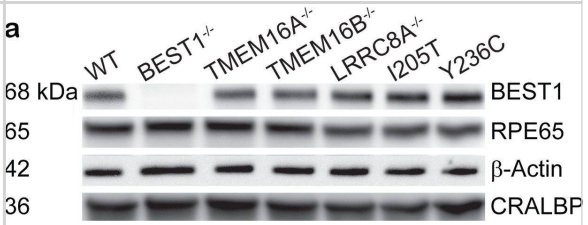


Simple Western: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - iPSC-RPE from all donors express RPE marker proteins. iPSC-RPE from the patient, the patients mom, the patients dad, and the three unrelated, unaffected controls all expressed RPE65. The negative control (iPSCs) had no detectable 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.

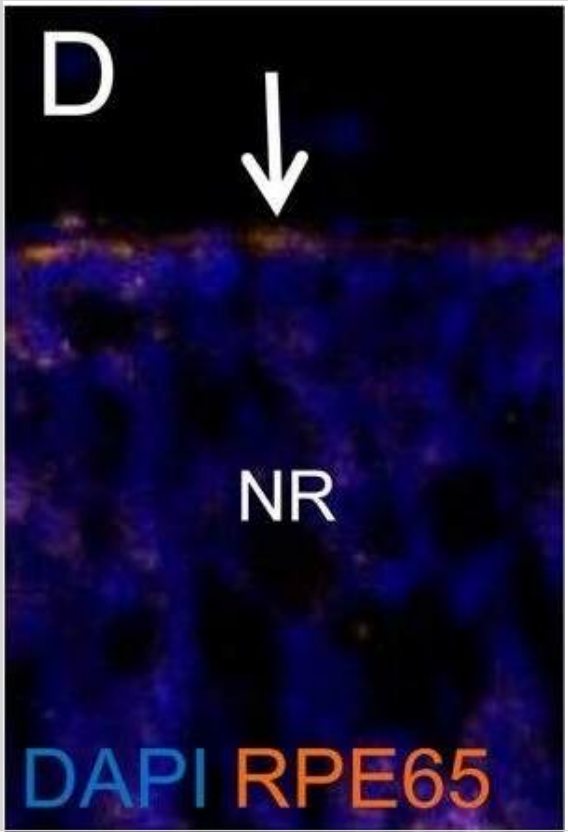


Western Blot: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - Immunoblotting showing the expression of RPE-specific proteins BEST1 (NB300-164), RPE65 (NB100-355), CRALBP, and the loading control beta-Actin in iPSC-RPE cells. Two gels/blots in the same panel were prepared from the same cell lysate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

Western Blot: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - 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: RPE65 Antibody (401.8B11.3D9) - BSA Free [NB100-355] - BMP5 induces ectopic RPE development in the central NR at optic cup stages. (A,B) Peripheral, untreated chick eye at E8 showing pigmentation & MITF expression being restricted to the outer layer of the eye. The arrowheads indicate the peripheral NR. (C-F) Higher magnification of the central chick eye at E8 showing the pigmented RPE, where RPE65 protein is detected (arrows). Faint Wnt2b & strong Vsx2 expression is detected in the central NR. The arrows indicate the RPE. (G,H) Following BMP5 application at E3.5/4, the NR detached (arrowhead) & MITF protein is now detected in the central NR (cNR). Note that a part of the cNR has RPE-like morphology (eRPE). (I-L) Higher magnification images of the eRPE. Ectopic pigment granulae, RPE65 protein & strong Wnt2b expression are detected in this region, whereas Vsx2 expression is downregulated (arrowheads). (M-O) Untreated chick eye at E8 showing pigmentation & MITF (arrow) & VSX2 protein distribution, restricted to the RPE & NR, respectively. (P-R) Following BMP5 application at E3.5/4, ectopic pigmentation & nuclear MITF protein are detected in the inner optic cup (arrowheads), whereas VSX2 protein is only weakly or not detected. The arrows indicate the RPE. (S) Graphical representation of the average thickness of the inner layer of the untreated (control) & BMP5-treated eye at E8. All expression pattern studies included n≥5. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28546339), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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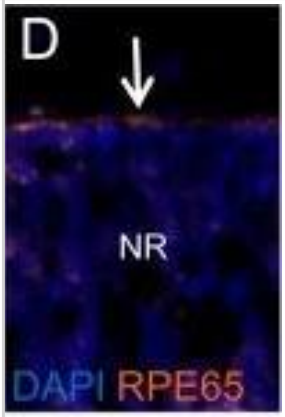

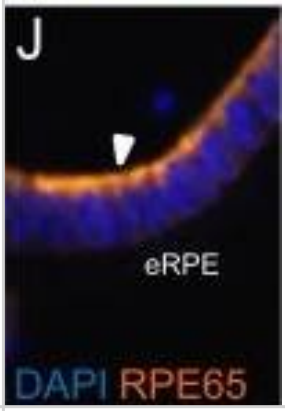

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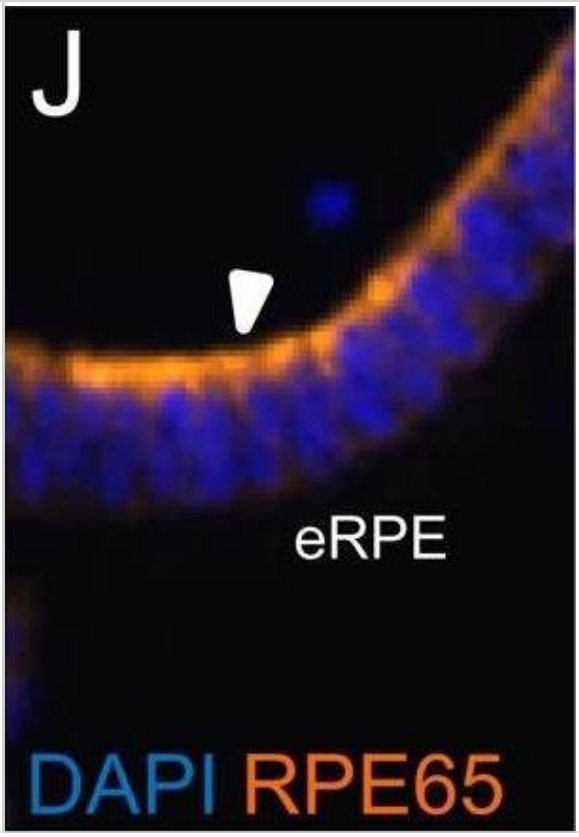

Publications

Thomas BB, Lin B, Martinez-Camarillo JC, Zhu D et Al. Co-grafts of Human Embryonic Stem Cell Derived Retina Organoids and Retinal Pigment Epithelium for Retinal Reconstruction in Immunodeficient Retinal Degenerate Royal College of Surgeons Rats Front Neurosci 2021-11-12 [PMID: 34764853]

Tan LX, Toops KA, Lakkaraju A. Protective responses to sublytic complement in the retinal pigment epithelium Proc. Natl. Acad. Sci. U.S.A. 2016-08-02 [PMID: 27432952]

Shechter Y, Cohen R, Namestnikov M et Al. Sequential Fabrication of a Three-Layer Retina-like Structure Gels 2024-05-15 [PMID: 38786253]

Z Geng, PJ Walsh, V Truong, C Hill, M Ebeling, RJ Kapphahn, SR Montezuma, C Yuan, H Roehrich, DA Ferrington, JR Dutton Generation of retinal pigmented epithelium from iPSCs derived from the conjunctiva of donors with and without age related macular degeneration PLoS ONE, 2017-03-10;12(3):e0173575. 2017-03-10 [PMID: 28282420]

Luo, M;Almeida, D;Dallacasagrande, V;Hedhli, N;Gupta, M;D'Amico, DJ;Kiss, S;Hajjar, KA; Annexin A2 promotes proliferative vitreoretinopathy in response to a macrophage inflammatory signal in mice Nature communications 2024-10-09 [PMID: 39384746]

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]

A Dhingra, JW Tobias, NJ Philp, K Boesze-Bat Transcriptomic Changes Predict Metabolic Alterations in LC3 Associated Phagocytosis in Aged Mice International Journal of Molecular Sciences, 2023-04-04;24(7):. 2023-04-04 [PMID: 37047689]

Chang YJ, Jenny LA, Li YS et al. CRISPR editing demonstrates rs10490924 raised oxidative stress in iPSC-derived retinal cells from patients with ARMS2/HTRA1-related AMD Proceedings of the National Academy of Sciences of the United States of America 2023-05-09 [PMID: 37126685]

Belinda J Hernandez, Nikolai P Skiba, Karolina Plössl, Madison Strain, Yutao Liu, Daniel Grigsby, Una Kelly, Martha A Cady, Vikram Manocha, Arvydas Maminishkis, TeddiJo Watkins, Sheldon S Miller, Allison Ashley-Koch, W Daniel Stamer, Bernhard H F Weber, Catherine Bowes Rickman, Mikael Klingeborn Polarized Desmosome and Hemidesmosome Shedding via Small Extracellular Vesicles is an Early Indicator of Outer Blood-Retina Barrier Dysfunction. Journal of extracellular biology 2023-10-01 [PMID: 38108061]

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] (Immunohistochemistry)

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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