Product Datasheet

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

Unit Size: 0.2 ml
Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

Reviews: 7  Publications: 66

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB100-355

Updated 9/23/2020 v.20.1

Earn rewards for product reviews and publications.
Submit a publication at www.novusbio.com/publications
Submit a review at www.novusbio.com/reviews/destination/NB100-355
RPE65 Antibody (401.8B11.3D9)

**Product Information**

<table>
<thead>
<tr>
<th>Item</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Size</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Aliquot and store at -20°C or -80°C. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>Clone</td>
<td>401.8B11.3D9</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.02% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG1 Kappa</td>
</tr>
<tr>
<td>Purity</td>
<td>Protein G purified</td>
</tr>
<tr>
<td>Buffer</td>
<td>PBS</td>
</tr>
<tr>
<td>Target Molecular Weight</td>
<td>65 kDa</td>
</tr>
</tbody>
</table>

**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**: Bovine RPE65 microsomal membrane proteins. [UniProt# Q28175]

**Product Application Details**

- **Applications**: Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vivo assay, CyTOF-ready, Immunofluorescence
- **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. Use in Immunoprecipitation reported by customer review. Use in In vivo reported in scientific literature (PMID:32173468).
Immunohistochemistry-Frozen: RPE65 Antibody (401.8B11.3D9) [NB100-355] - Staining of RPE65 in a cryosection of mouse retina tissue using RPE65 antibody (clone 401.8B11.3D9).

Western Blot: RPE65 Antibody (401.8B11.3D9) [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) [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.

Western Blot: RPE65 Antibody (401.8B11.3D9) [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 licence.
Immunocytochemistry/Immunofluorescence: RPE65 Antibody (401.8B11.3D9) [NB100-355] - ARPE-19 monolayer on glass substrate. Fixative Methanol Blocking Goat Serum. Dilution 1:250. Incubation 1h RT. This image was submitted via customer Review.

Immunohistochemistry-Paraffin: RPE65 Antibody (401.8B11.3D9) [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).

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

Mitrousis N, Hacibekiroglu S, Ho M et al. Hydrogel-mediated co-transplantation of retinal pigmented epithelium and photoreceptors restores vision in an animal model of advanced retinal degeneration Biomaterials Jul 1 2020 12:00AM [PMID: 32791386] (IHC, Mouse)


Details:
The primate species used as part of this study included two cynomolgus and one macaque.

Gardiner KL, Cideciyan AV, Swider M et al. Long-Term Structural Outcomes of Late-Stage RPE65 Gene Therapy Mol. Ther. 2019 Sep 03 [PMID: 31604676] (IHC, Canine)


Details:
Citation using the Alexa Fluor 488 version of this antibody.

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 instructi
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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NB100-355

Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications