abcam

Product datasheet

Anti-RPE65 antibody [EPR22579-44] ab231782

リコンビナント RabMAb

5 References 画像数 10

製品の概要

製品名	Anti-RPE65 antibody [EPR22579-44]
製品の詳細	Rabbit monoclonal [EPR22579-44] to RPE65
由来種	Rabbit
アプリケーション	**適用あり:** IHC-P, WB, IHC-Fr, IP, mIHC
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: Human eye tissue lysate; mouse eye tisue lysate; rat eye tissue lysate. IHC-P: Human retina tissue; mouse retina tissue; rat retina tissue. IHC-Fr: Mouse retina tissue; rat retina tissue. IP: Mouse eyeball lysate. mIHC: Human retina tissue.
特記事項	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR22579-44

アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab231782の使用に適用されます**

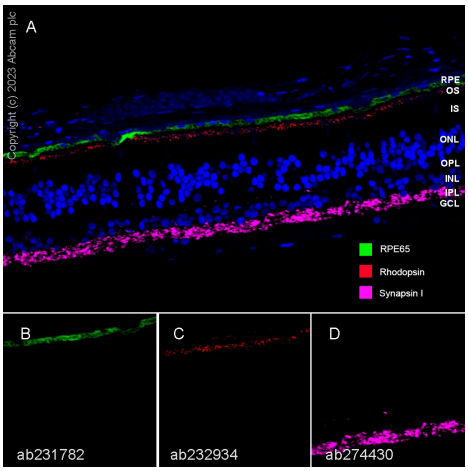
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 65 kDa (predicted molecular weight: 61 kDa).
IHC-Fr		1/250. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).
IP		1/30.
mIHC		Use at an assay dependent concentration.

ターゲット情報

機能	Plays important roles in the production of 11-cis retinal and in visual pigment regeneration. The soluble form binds vitamin A (all-trans-retinol), making it available for LRAT processing to all-trans-retinyl ester. The membrane form, palmitoylated by LRAT, binds all-trans-retinyl esters, making them available for IMH (isomerohydrolase) processing to all-cis-retinol. The soluble form is regenerated by transferring its palmitoyl groups onto 11-cis-retinol, a reaction catalyzed by LRAT. The enzymatic activity is linearly dependent of the expression levels and membrane association.
組織特異性	Retinal pigment epithelium specific.
関連疾患	Defects in RPE65 are the cause of Leber congenital amaurosis type 2 (LCA2) [MIM:204100]. LCA designates a clinically and genetically heterogeneous group of childhood retinal degenerations, generally inherited in an autosomal recessive manner. Affected infants have little or no retinal photoreceptor function as tested by electroretinography. LCA represents the most common genetic cause of congenital visual impairment in infants and children. Defects in RPE65 are the cause of retinitis pigmentosa type 20 (RP20) [MIM:613794]. RP leads to degeneration of retinal photoreceptor cells. Patients typically have night vision blindness and loss of midperipheral visual field. As their condition progresses, they lose their far peripheral visual field and eventually central vision as well. RP20 inheritance is autosomal dominant.
配列類似性	Belongs to the carotenoid oxygenase family.
翻訳後修飾	Palmitoylation by LRAT regulates ligand binding specificity; the palmitoylated form (membrane form) specifically binds all-trans-retinyl-palmitate, while the soluble unpalmitoylated form binds all-trans-retinol (vitamin A).
細胞内局在	Cytoplasm. Cell membrane. Attached to the membrane by a lipid anchor when palmitoylated (membrane form), soluble when unpalmitoylated.

画像



Multiplex immunohistochemistry - Anti-RPE65 antibody [EPR22579-44] (ab231782)

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Human retina tissue labeling RPE65, Rhodopsin and Synapsin I with ab231782 at 1/8000 dilution, **ab232934** at 1/8000 dilution and **ab274430** at 1/1500 dilution followed by a ready to use Opal Polymer HRP Ms + Rb secondary antibody. Nuclear counter stain used was DAPI.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

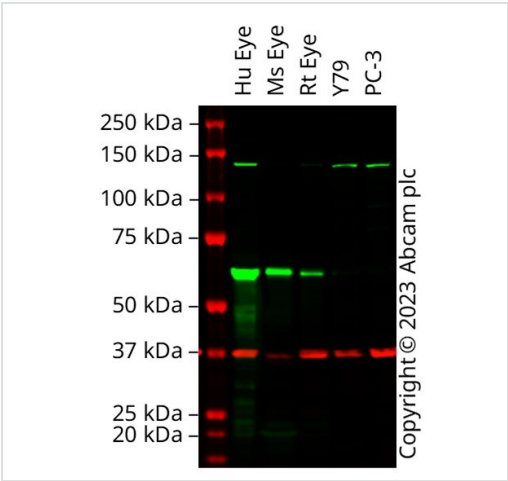
Panel A: merged staining of anti-Synapsin I (magenta; Opal™690), anti-RPE65 (green; Opal™520) and anti-Rhodopsin (red; Opal™570) on human retina.
Panel B: anti-RPE65 stained on pigmented layer.
Panel C: anti-Rhodopsin stained on rod photoreceptor cells.
Panel D: anti-Synapsin I stained on inner plexiform layer.

The section was incubated in three rounds of staining: in the order of **ab274430**, ab231782, and **ab232934** for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

The section was incubated in three rounds of staining: in the order of **ab312840**, **ab16669**, and **ab236434** for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Western blot - Anti-RPE65 antibody [EPR22579-44] (ab231782)

All lanes : Anti-RPE65 antibody [EPR22579-44] (ab231782) at 1/1000 dilution

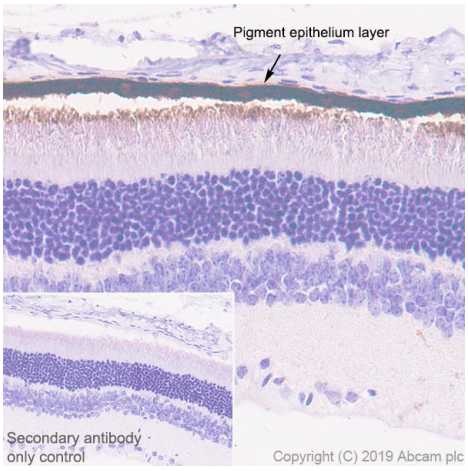
Lane 1 : Human Eye cell lysate
Lane 2 : Mouse Eye cell lysate
Lane 3 : Rat Eye cell lysate
Lane 4 : Y79 cell lysate
Lane 5 : PC-3 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 61 kDa
Observed band size: 65 kDa

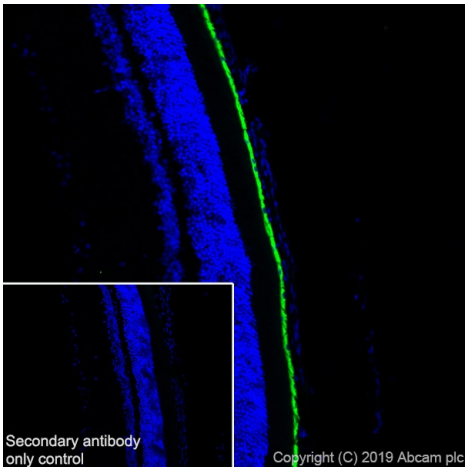
False colour image of Western blot: Anti-RPE65 antibody [EPR22579-44] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab231782 was shown to bind specifically to RPE65. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RPE65 antibody [EPR22579-44] (ab231782)

Immunohistochemical analysis of paraffin-embedded mouse retina tissue labeling RPE65 with ab231782 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Cytoplasmic staining on pigment epithelium cells of mouse retina (PMID: 25941382, PMID: 22238088 is observed. Counter stained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Frozen sections) - Anti-RPE65 antibody [EPR22579-44] (ab231782)

Immunohistochemical analysis of frozen section of 4%PFA-fixed, 0.2% Triton X-100 permeabilized rat retinal tissue labeling RPE65 with ab231782 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution (green). Positive staining on retinal pigment epithelium (PMID: 17848510) is observed. The nuclear counter stain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.

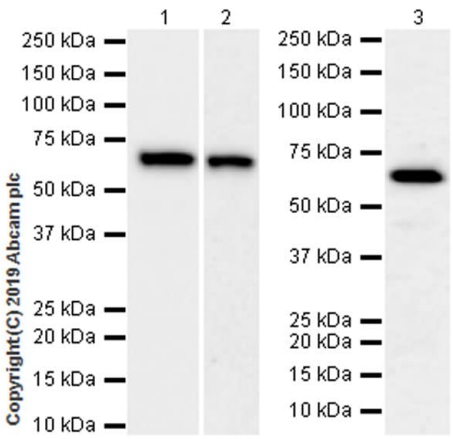
Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).

Immunoprecipitation - Anti-RPE65 antibody [EPR22579-44] (ab231782)

RPE65 was immunoprecipitated from 0.35 mg of mouse eyeball lysate with ab231782 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab231782 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used as secondary antibody at 1/5000 dilution.

Lane 1: Mouse eyeball lysate 10 µg (Input).
Lane 2: ab231782 IP in mouse eyeball lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab231782 in mouse eyeball lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 3 minutes.



Western blot - Anti-RPE65 antibody [EPR22579-44] (ab231782)

All lanes : Anti-RPE65 antibody [EPR22579-44] (ab231782) at 1/1000 dilution

Lane 1 : Human eye tissue lysate
Lane 2 : Mouse eye tissue lysate
Lane 3 : Rat eye tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

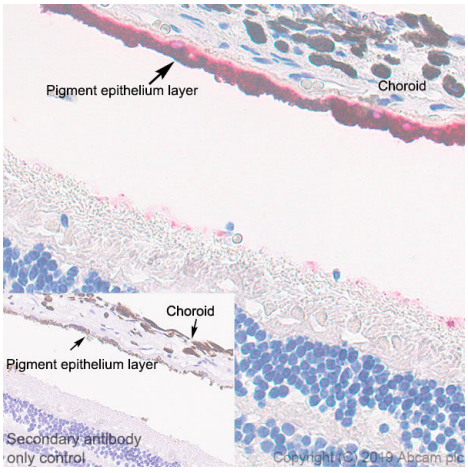
Predicted band size: 61 kDa
Observed band size: 65 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure times.

Lane 1: 26 seconds. Lanes 2 & 3: 3 minutes.

The molecular weight observed is consistent with what has been described in the literature (PMID:17848510).



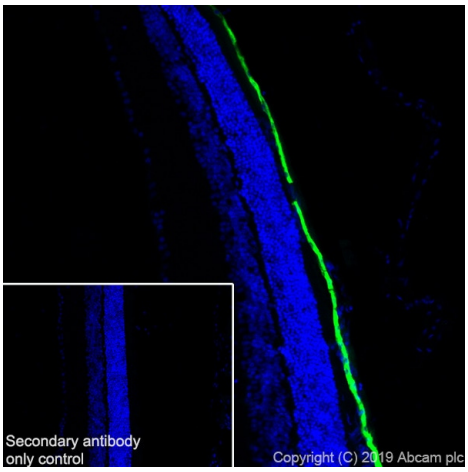
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RPE65 antibody [EPR22579-44] (ab231782)

Immunohistochemical analysis of paraffin-embedded human retina tissue labeling RPE65 with ab231782 at 1/4000 dilution, followed by ready to use AP-labeled secondary antibody kit. Cytoplasmic staining on pigment epithelium cells of human retina (PMID: 25941382; PMID: 22238088) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use AP-labeled secondary antibody kit.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab231782 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



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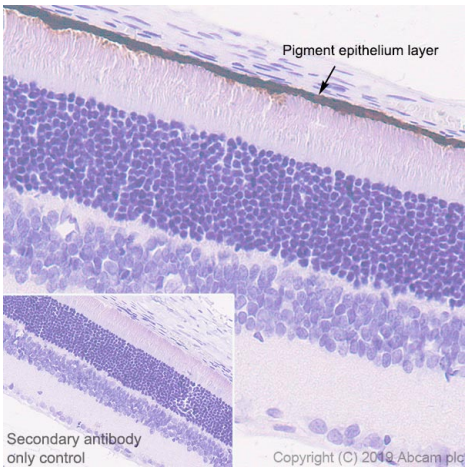

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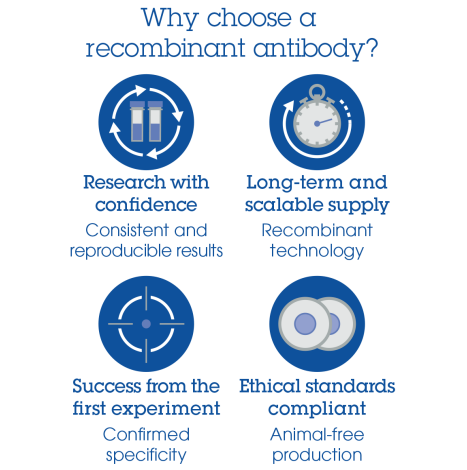
Immunohistochemical analysis of paraffin-embedded rat retina tissue labeling RPE65 with ab231782 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Cytoplasmic staining on pigment epithelium cells of rat retina (PMID: 25941382, PMID: 22238088) is observed. Counter stained with hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Anti-RPE65 antibody [EPR22579-44] (ab231782)

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