abcam

Product datasheet

Anti-Optineurin antibody [EPR20654] ab213556





重组 RabMAb

15 图像 **5 References**

概述

产品名称 Anti-Optineurin抗体[EPR20654]

描述 兔单克隆抗体[EPR20654] to Optineurin

宿主 Rabbit

经测试应用 适用于: IP, IHC-P, WB, IHC-Fr

不适用于: ICC/IF

种属反应性 与反应: Mouse, Rat, Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

阳性对照 WB: Human fetal brain, fetal kidney, placenta and skeletal muscle lysates; Rat brain, retina,

> placenta and heart lysates; Mouse retina and placenta lysates; 293T, U-2 OS and NIH/3T3 whole cell lysates. IHC-P: Human retina and cerebrum tissues; Mouse retina and cerebrum tissues; Rat

cerebellum tissue. IP: 293T and U-2 OS whole cell lysate.

常规说明 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**.

性能

形式 Liquid

存放说明 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.

存储溶液 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

纯度 Protein A purified

单克隆 克隆

克隆编号 EPR20654

同种型 IgG

应用

The Abpromise guarantee

Abpromise™承诺保证使用ab213556于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

| 应用 | Ab评论 | 说明 |
|--------|------|---------------------------------------------------------------------------------------------------------------------------|
| IP | | 1/40. |
| IHC-P | | 1/500. 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 68 kDa (predicted molecular weight: 66 kDa). |
| IHC-Fr | | 1/100. |

应用说明

Is unsuitable for ICC/IF.

靶标

功能

Plays an important role in the maintenance of the Golgi complex, in membrane trafficking, in exocytosis, through its interaction with myosin VI and Rab8. Links myosin VI to the Golgi complex and plays an important role in Golgi ribbon formation. Negatively regulates the induction of IFNB in response to RNA virus infection. Plays a neuroprotective role in the eye and optic nerve. Probably part of the TNF-alpha signaling pathway that can shift the equilibrium toward induction of cell death. May act by regulating membrane trafficking and cellular morphogenesis via a complex that contains Rab8 and hungtingtin (HD). May constitute a cellular target for adenovirus E3 14.7, an inhibitor of TNF-alpha functions, thereby affecting cell death.

组织特异性

Present in acqueous humor of the eye (at protein level). Highly expressed in trabecular meshwork. Expressed nonpigmented ciliary epithelium, retina, brain, adrenal cortex, fetus, lymphocyte, fibroblast, skeletal muscle, heart, liver, brain and placenta.

疾病相关

Defects in OPTN are the cause of primary open angle glaucoma type 1E (GLC1E) [MIM:137760]. Primary open angle glaucoma (POAG) is characterized by a specific pattern of optic nerve and visual field defects. The angle of the anterior chamber of the eye is open, and usually the intraocular pressure is increased. The disease is asymptomatic until the late stages, by which time significant and irreversible optic nerve damage has already taken place.

Defects in OPTN are a cause of susceptibility to normal pressure glaucoma (NPG) [MIM:606657]. Defects in OPTN are the cause of amyotrophic lateral sclerosis type 12 (ALS12) [MIM:613435]. It is a neurodegenerative disorder affecting upper motor neurons in the brain and lower motor neurons in the brain stem and spinal cord, resulting in fatal paralysis. Sensory abnormalities are absent. Death usually occurs within 2 to 5 years. The etiology of amyotrophic lateral sclerosis is likely to be multifactorial, involving both genetic and environmental factors. The disease is inherited in 5-10% of the cases.

结构域

Ubiquitin-binding motif (UBAN) is essential for its inhibitory function, subcellular localization and interaction with TBK1.

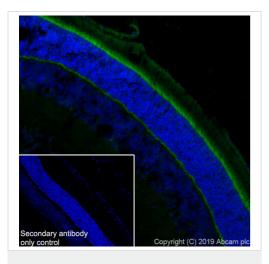
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细胞定位

Phosphorylated. Phosphorylation is induced by phorbol esters and decreases its half-time.

Cytoplasm > perinuclear region. Golgi apparatus. Golgi apparatus > trans-Golgi network. Found in the perinuclear region and associates with the Golgi apparatus. Colocalizes with MYO6 and RAB8 at the Golgi complex and in vesicular structures close to the plasma membrane.

图片

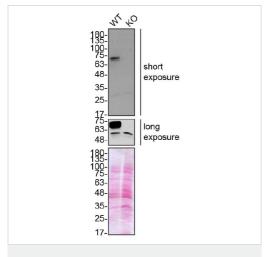


Immunohistochemistry (Frozen sections) - Anti-Optineurin antibody [EPR20654] (ab213556) ab213556 staining Optineurin in Mouse retina tissue sections by Immunohistochemistry (IHC-Fr - frozen sections). Tissue was fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton.

Samples were incubated with primary antibody (1/100). An Alexa Fluor[®] 488 Goat anti-Rabbit secondary (1/1000) was used as the secondary antibody. Counter stained with DAPI.

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

Positive staining on mouse retina (PMID: 15607428).



Western blot - Anti-Optineurin antibody [EPR20654] (ab213556)

All lanes : Anti-Optineurin antibody [EPR20654] (ab213556) at 1/5000 dilution

Lane 1: Wild-type U-2 OS cell lysate

Lane 2: OPTN knockout U-2 OS cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 66 kDa

ab213556 was shown to react with OPTN in wild-type U-2 OS cells in Western blot with loss of signal observed in a OPTN knockout cell line. Wild-type U-2 OS and OPTN knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab213556 overnight at 4 °C at a 1/5000 dilution. Blots were incubated with goat anti-rabbit HRP secondary antibodies at 1/5000 before imaging. These data were provided by YCharOS Inc., an open science company with the

mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.

Secondary antibody only control Copyright (C) 2017 Abcam plic

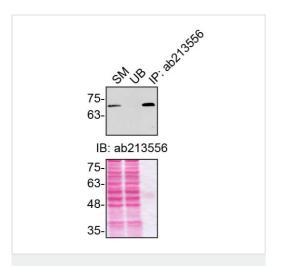
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Optineurin antibody
[EPR20654] (ab213556)

Immunohistochemical analysis of paraffin-embedded human retina tissue labeling Optineurin with ab213556 at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) Ready to use. Cytoplasmic staining on human retina (PMID: 15607428).

Counter stained with Hematoxylin.

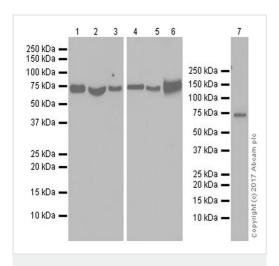
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-Optineurin antibody [EPR20654] (ab213556)

Immunoprecipitation of OPTN in c-43 cells. Lysates were prepared and immunoprecipitation was performed using 1.0 µg of ab213556 pre-coupled to prot.A-Sepharose beads. Samples were washed and processed for western blot with ab213556 at 1/5000. This data was kindly provided by the YCharOS Inc., an open science company with the mission of characterizing every commercially available antibody reagent. Abcam are working with YCharOS to support their mission of antibody characterisation using knock out cell lines.



Western blot - Anti-Optineurin antibody [EPR20654] (ab213556)

All lanes : Anti-Optineurin antibody [EPR20654] (ab213556) at 1/1000 dilution

Lane 1: Human skeletal muscle lysate at 20 µg

Lane 2: 293T (Human epithelial cell line from embryonic kidney)

whole cell lysate at 20 µg

Lane 3: Rat brain lysate at 20 µg

Lane 4: Mouse retina lysate at 20 µg

Lane 5: Rat retina lysate at 20 µg

Lane 6: Rat placenta lysate at 20 µg

Lane 7: NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell

lysate at 10 µg

Secondary

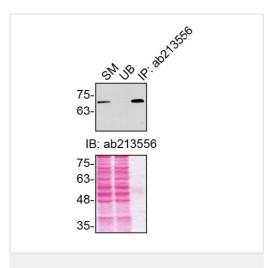
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 66 kDa
Observed band size: 68 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

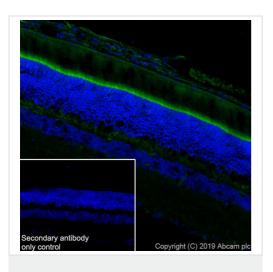
Exposure time: Lane 1-3/7: 3 minutes; Lane 4-6: 10 seconds.

The MW observed is consistent with the literature (PMID 20174559, PMID 24983867, PMID 11834836).



Immunoprecipitation - Anti-Optineurin antibody [EPR20654] (ab213556)

Immunoprecipitation of OPTN in U-2 OS cells. Lysates were prepared and immunoprecipitation was performed using 1.0 μ g of ab213556 pre-coupled to prot.A-Sepharose beads. Samples were washed and processed for western blot with ab213556 at 1/5000. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate. These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.

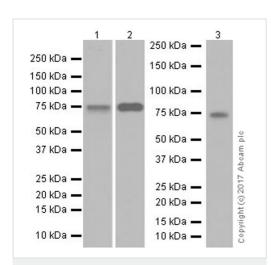


Immunohistochemistry (Frozen sections) - Anti-Optineurin antibody [EPR20654] (ab213556)

ab213556 staining Optineurin in Rat retina tissue sections by Immunohistochemistry (IHC-Fr - frozen sections). Tissue was fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton. Samples were incubated with primary antibody (1/100). An Alexa Fluor[®] 488 Goat anti-Rabbit secondary (1/1000) was used as the secondary antibody. Counter stained with DAPI.

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

Positive staining on rat retina (PMID: 15607428).



Western blot - Anti-Optineurin antibody [EPR20654] (ab213556)

All lanes : Anti-Optineurin antibody [EPR20654] (ab213556) at 1/1000 dilution

Lane 1 : Human fetal brain lysate

Lane 2 : Human fetal kidney lysate

Lane 3 : Human placenta lysate

Lysates/proteins at 10 µg per lane.

Secondary

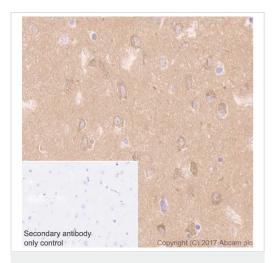
All lanes : VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/4000 dilution

Predicted band size: 66 kDa Observed band size: 68 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 3 seconds; Lane 2/3: 30 seconds.

The MW observed is consistent with the literature (PMID 20174559, PMID 24983867, PMID 11834836).



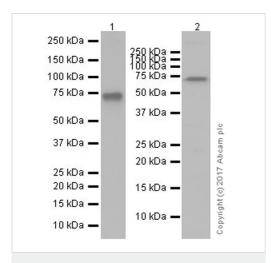
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Optineurin antibody
[EPR20654] (ab213556)

Immunohistochemical analysis of paraffin-embedded human cerebrum tissue labeling Optineurin with ab213556 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on human cerebrum (PMID: 26303227).

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.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Optineurin antibody [EPR20654] (ab213556)

All lanes : Anti-Optineurin antibody [EPR20654] (ab213556) at 1/1000 dilution

Lane 1: Rat heart lysate

Lane 2: Mouse placenta lysate

Lysates/proteins at 10 µg per lane.

Secondary

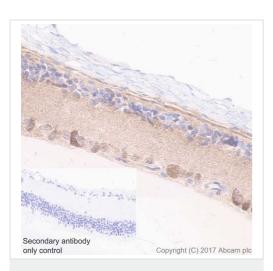
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 66 kDa Observed band size: 68 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 3 minutes; Lane 2: 10 seconds.

The MW observed is consistent with the literature (PMID 20174559, PMID 24983867, PMID 11834836).



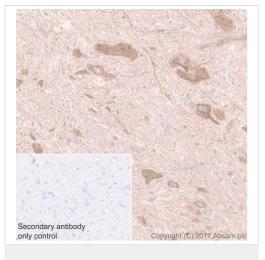
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Optineurin antibody
[EPR20654] (ab213556)

Immunohistochemical analysis of paraffin-embedded mouse retina tissue labeling Optineurin with ab213556 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on mouse retina (PMID: 15607428).

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



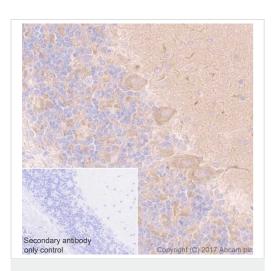
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Optineurin antibody
[EPR20654] (ab213556)

Immunohistochemical analysis of paraffin-embedded mouse cerebrum tissue labeling Optineurin with ab213556 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on mouse cerebrum (PMID: 26303227).

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



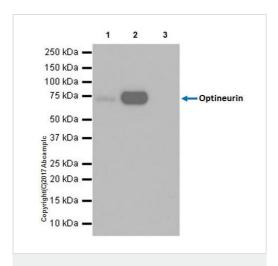
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Optineurin antibody
[EPR20654] (ab213556)

Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue labeling Optineurin with ab213556 at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) Ready to use. Cytoplasmic staining on rat cerebellum (PMID: 26303227).

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-Optineurin antibody [EPR20654] (ab213556)

Optineurin was immunoprecipitated from 0.35 mg of 293T (Human epithelial cell line from embryonic kidney) whole cell lysate with ab213556 at 1/40 dilution. Western blot was performed from the immunoprecipitate using ab213556 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution

Lane 1: 293T whole cell lysate 10 µg (Input).

Lane 2: ab213556 IP in 293T whole cell lysate.

Lane 3: Rabbit monoclonal lgG (ab172730) instead of ab213556 in 293T whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.



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