

Product datasheet

Anti-Optineurin antibody [EPR20654] ab213556

KO VALIDATED Recombinant RabMAB

[1 References](#) [15 Images](#)

Overview

Product name	Anti-Optineurin antibody [EPR20654]
Description	Rabbit monoclonal [EPR20654] to Optineurin
Host species	Rabbit
Tested applications	Suitable for: IP, IHC-P, WB, IHC-Fr Unsuitable for: ICC/IF
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	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.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAB[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAB[®] patents.</p>

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number EPR20654

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab213556 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
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.

Application notes Is unsuitable for ICC/IF.

Target

Function 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 hungtingin (HD). May constitute a cellular target for adenovirus E3 14.7, an inhibitor of TNF-alpha functions, thereby affecting cell death.

Tissue specificity Present in aqueous 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.

Involvement in disease 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.

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

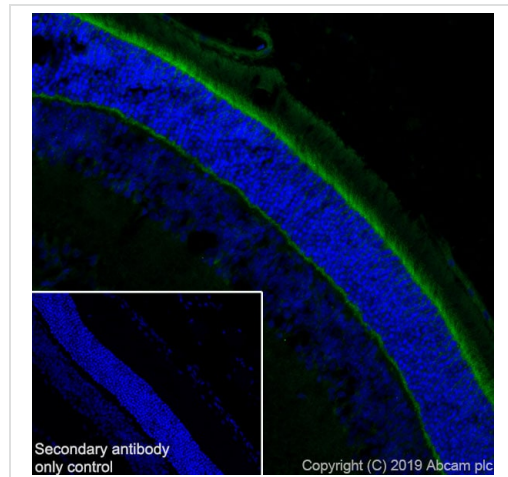
Post-translational modifications

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

Cellular localization

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.

Images

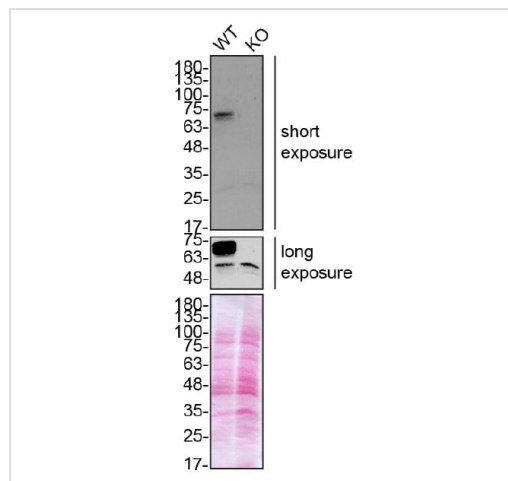


ab213556 staining Optineurin in Mouse retina tissue sections by Immunohistochemistry (IHC-Fr - frozen sections). Tissue was fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton. Samples were incubated with primary antibody (1/100). An Alexa Fluor® 488 Goat anti-Rabbit secondary (1/1000) was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

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



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

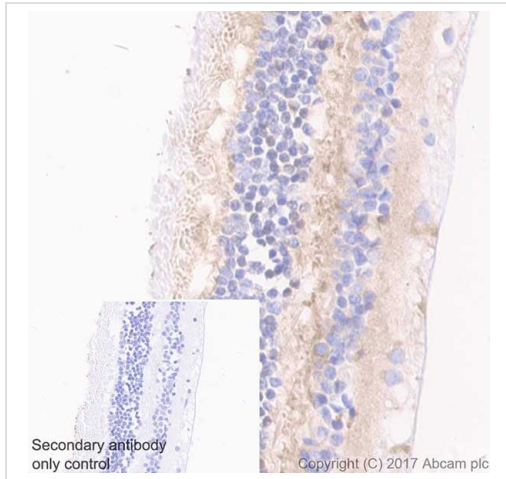
Lysates/proteins at 20 µg per lane.

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.



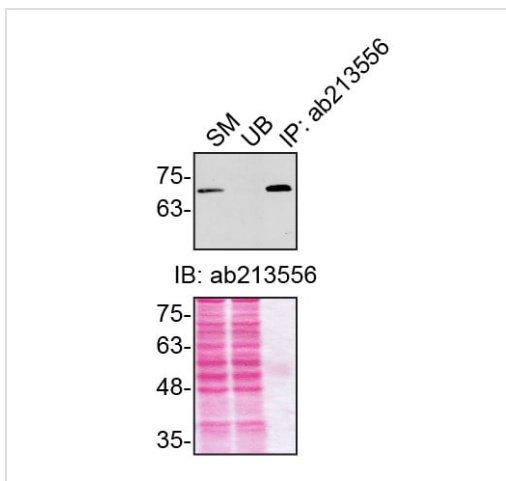
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Optineurin antibody [EPR20654] (ab213556)

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

Counter stained with Hematoxylin.

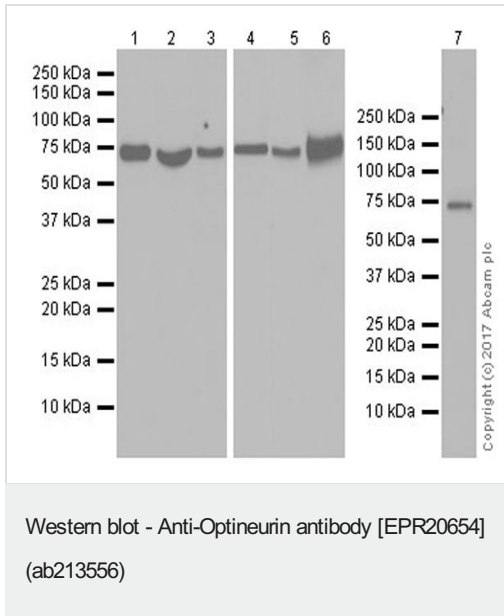
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

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



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

Immunoprecipitation of OPTN in c-43 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. 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.



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 IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

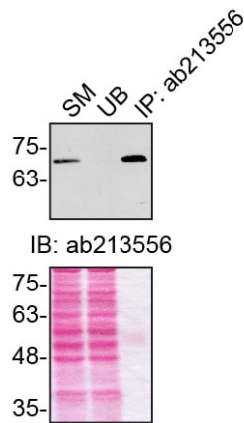
Predicted band size: 66 kDa

Observed band size: 68 kDa

Blocking/Dilution buffer: 5% NFDm/TBST.

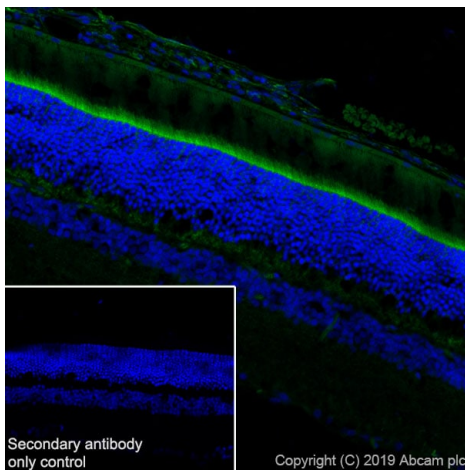
Exposure time: Lane 1-3/7: 3 minutes; Lane 4-6: 10 seconds.

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 µg 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.

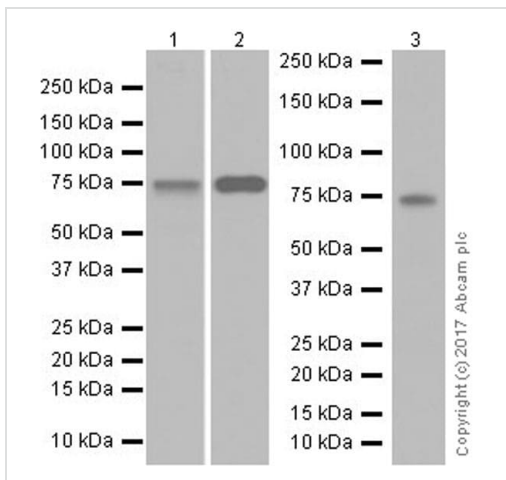


Immunohistochemistry (Frozen sections) - Anti-Optineurin antibody [EPR20654] (ab213556)

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) ([ab131366](#)) at 1/4000 dilution

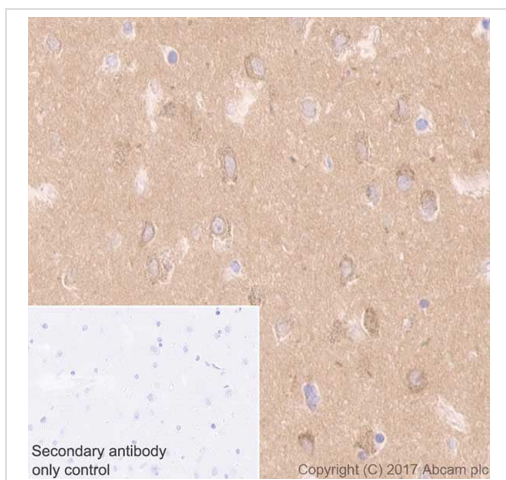
Predicted band size: 66 kDa

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).



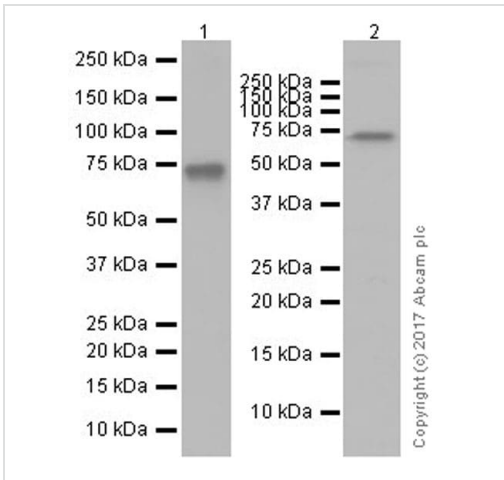
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Optineurin antibody [EPR20654] (ab213556)

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 IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

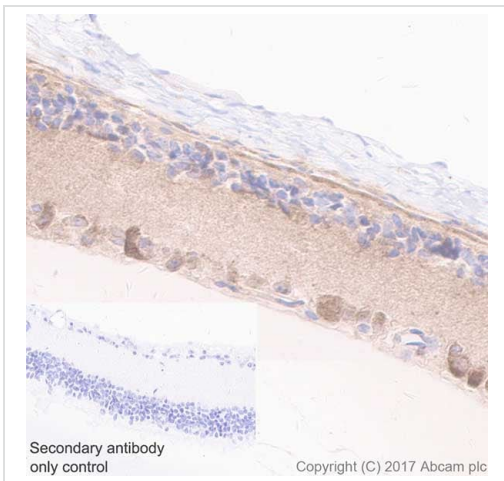
Predicted band size: 66 kDa

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).



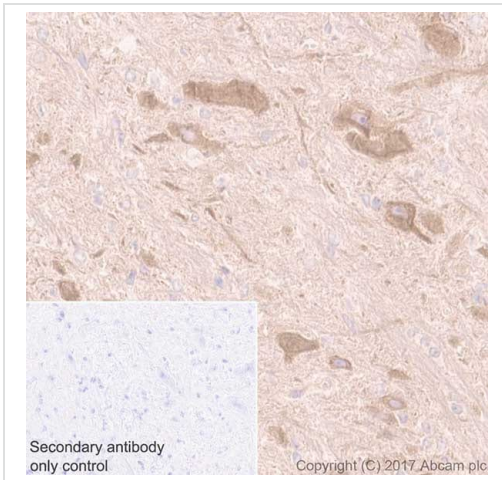
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Optineurin antibody [EPR20654] (ab213556)

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 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.



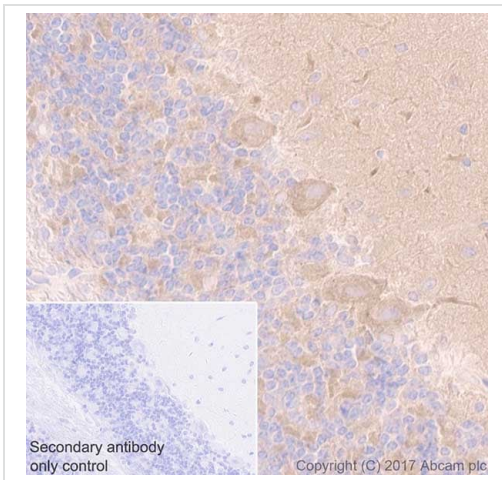
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Optineurin antibody [EPR20654] (ab213556)

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 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.



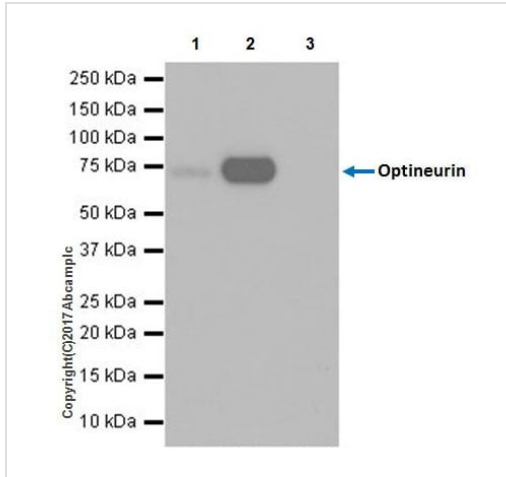
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Optineurin antibody [EPR20654] (ab213556)

Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue labeling Optineurin with ab213556 at 1/500 dilution, followed by Goat Anti-Rabbit IgG 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 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.



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 IgG ([ab172730](#)) instead of ab213556 in 293T whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFD/MBST.

Exposure time: 3 minutes.

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-Optineurin antibody [EPR20654] (ab213556)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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