abcam

Product datasheet

Anti-Alpha-synuclein antibody [MJFR1] ab138501

敲除验证 重组 RabMAb

★★★★★ 9 Abreviews 118 References 13 图像

概述

产品名称	Anti-Alpha-synuclein抗体[MJFR1]
描述	兔单克隆抗体[MJFR1] to Alpha-synuclein
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, IHC-P, IP, ICC/IF
种属反应性	**与反应:** Human
免疫原	Recombinant full length protein within Human Alpha-synuclein aa 1 to the C-terminus. The exact sequence is proprietary. Database link: **P37840**
表位	The epitope was mapped to amino acids 118-123 (VDPDNE).
阳性对照	WB: Recombinant Human Alpha-synuclein protein (**ab51189**), HAP1 and HEK293-T cell lysate; Human brain whole cell lysate; Human brain tissue lysate. IHC-P: FFPE Human Normal Cerebral Cortex; FFPE Human Normal and Parkinson Substantia Nigra tissue. Flow Cyt (intra): Hap1 cells. ICC/IF: Hap1 and U87-MG cells.
常规说明	Alpha-Synuclein is expressed predominantly in the brain, where it is concentrated in presynaptic nerve terminals. The deposition of the abundant presynaptic brain protein alpha-synuclein as fibrillary aggregates in neurons or glial cells is a hallmark lesion in a subset of neurodegenerative disorders. These disorders include Parkinson's disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy, collectively referred to as synucleinopathies. Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the progressive accumulation in selected neurons of protein inclusions containing alpha-synuclein and ubiquitin. This antibody was developed with support from The Michael J. Fox Foundation, in collaboration with the laboratory of Dr. Michael Schlossmacher (University of Ottawa). Abcam recommended secondaries - Goat Anti-Rabbit HRP (**ab205718**) and Goat Anti-Rabbit Alexa Fluor® 488 (**ab150077**). Or search our wide range of secondary antibodies for use with your experiment. 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**.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

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. Avoid freeze / thaw cycle.
存储溶液	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
纯度	Protein A purified
克隆	单克隆
克隆编号	MJFR1
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab138501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
Flow Cyt (Intra)		1/200. For unpurified use at 1/20. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ **(7)**	1/10000. Predicted molecular weight: 14 kDa. **For unpurified use at 1/1000.00000 - 1/10000.00000**
IHC-P		1/150. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. **For unpurified use at 1/15 to 1/300.** See **IHC antigen retrieval protocols**.
IP		1/600. **For unpurified use at 1/50**
ICC/IF	★★★★★ **(1)**	Use a concentration of 0.1 µg/ml.

靶标

功能	May be involved in the regulation of dopamine release and transport. Induces fibrillization of microtubule-associated protein tau. Reduces neuronal responsiveness to various apoptotic stimuli, leading to a decreased caspase-3 activation.
组织特异性	Expressed principally in brain but is also expressed in low concentrations in all tissues examined

except in liver. Concentrated in presynaptic nerve terminals.

疾病相关

Genetic alterations of SNCA resulting in aberrant polymerization into fibrils, are associated with several neurodegenerative diseases (synucleinopathies). SNCA fibrillar aggregates represent the major non A-beta component of Alzheimer disease amyloid plaque, and a major component of Lewy body inclusions. They are also found within Lewy body (LB)-like intraneuronal inclusions, glial inclusions and axonal spheroids in neurodegeneration with brain iron accumulation type 1.
Parkinson disease 1
Parkinson disease 4
Dementia Lewy body

序列相似性

Belongs to the synuclein family.

结构域

The 'non A-beta component of Alzheimer disease amyloid plaque' domain (NAC domain) is involved in fibrils formation. The middle hydrophobic region forms the core of the filaments. The C-terminus may regulate aggregation and determine the diameter of the filaments.

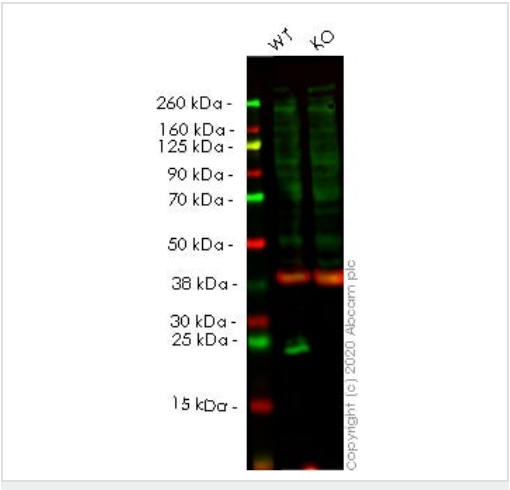
翻译后修饰

Phosphorylated, predominantly on serine residues. Phosphorylation by CK1 appears to occur on residues distinct from the residue phosphorylated by other kinases. Phosphorylation of Ser-129 is selective and extensive in synucleinopathy lesions. In vitro, phosphorylation at Ser-129 promoted insoluble fibril formation. Phosphorylated on Tyr-125 by a PTK2B-dependent pathway upon osmotic stress.
Hallmark lesions of neurodegenerative synucleinopathies contain alpha-synuclein that is modified by nitration of tyrosine residues and possibly by dityrosine cross-linking to generated stable oligomers.
Ubiquitinated. The predominant conjugate is the diubiquitinated form.
Acetylation at Met-1 seems to be important for proper folding and native oligomeric structure.

细胞定位

Cytoplasm, cytosol. Membrane. Nucleus. Cell junction, synapse. Secreted. Membrane-bound in dopaminergic neurons.

图片



Western blot - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

All lanes : Anti-Alpha-synuclein antibody [MJFR1] (ab138501) at 1/10000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : SNCA knockout HEK-293T cell lysate

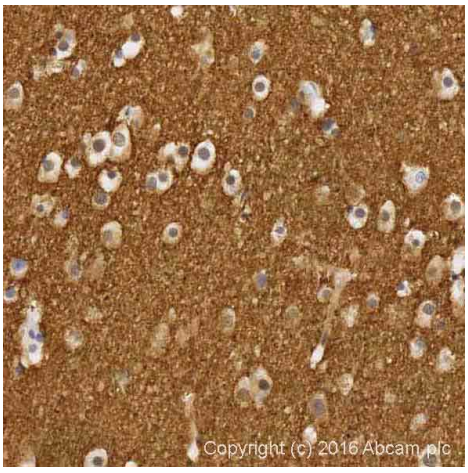
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 14 kDa
Observed band size: 18 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab138501 observed at 18 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

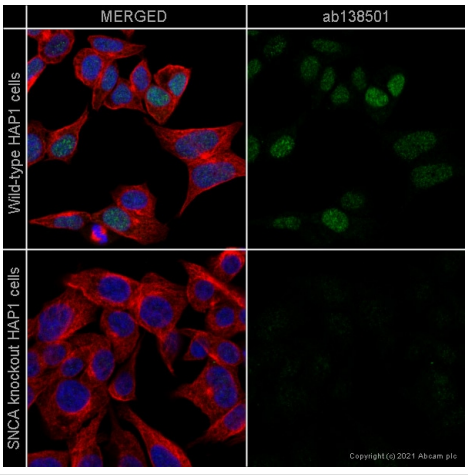
ab138501 was shown to react with SNCA in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab255433** (knockout cell lysate **ab263769**) was used. Wild-type HEK-293T and SNCA knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab138501 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

IHC image of alpha Synuclein staining in normal Human cerebral cortex formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab138501, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

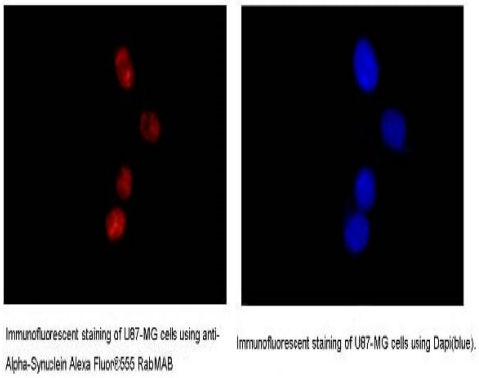
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry/ Immunofluorescence - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

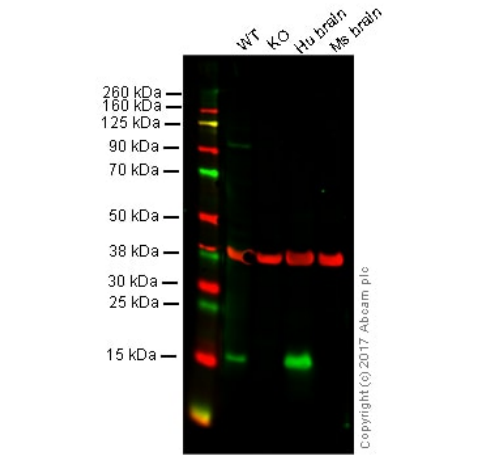
ab138501 staining Alpha-Synuclein in wild-type Hap1 cells (top panel) and SNCA knockout Hap1 cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab138501 at 0.1 µg/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems

TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

Immunocytochemistry/Immunofluorescence analysis of U87-MG (Human glioblastoma-astrocytoma epithelial cell line) cells labeling alpha Synuclein with purified ab138501 at 1/150 dilution (left panel). Cells were fixed with 4% paraformaldehyde. A Goat anti rabbit IgG(Alexa Fluor®555) (1/200) was used as the secondary antibody. DAPI (blue) was used as the nuclear counter stain (right panel).



Western blot - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

All lanes : Anti-Alpha-synuclein antibody [MJFR1] (ab138501) at 1/10000 dilution

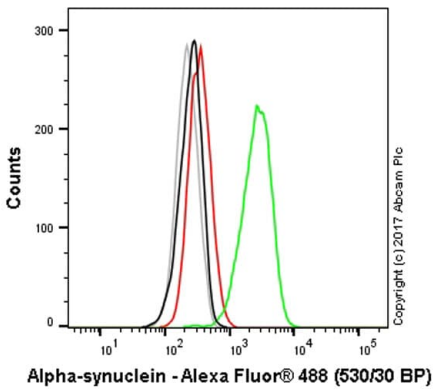
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : SNCA knockout HAP1 whole cell lysate
Lane 3 : Human brain whole cell lysate
Lane 4 : Mouse brain whole cell lysate

Lysates/proteins at 40 µg per lane.

Predicted band size: 14 kDa

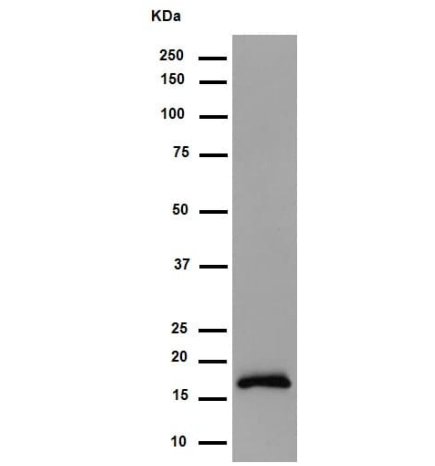
Lanes 1 - 4: Merged signal (red and green). Green - ab138501 observed at 14 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab138501 was shown to specifically react with SNCA in wild-type HAP1 cells. No band was observed when SNCA knockout samples were used. Wild-type and SNCA knockout samples were subjected to SDS-PAGE. Ab138501 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/10000 and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-SNCA knockout cells (red line) stained with ab138501. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab138501, 1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) presorbed (**ab150081**) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-SNCA knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.



Western blot - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

Anti-Alpha-synuclein antibody [MJFR1] (ab138501) at 1/10000 dilution (purified) + Human fetal brain at 20 µg

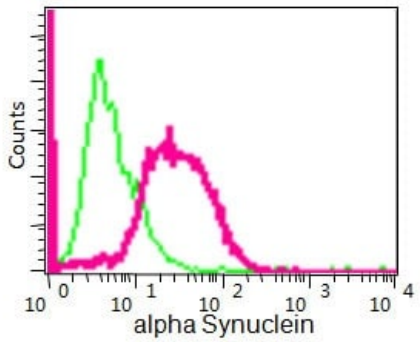
Secondary

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 14 kDa

Observed band size: 18 kDa

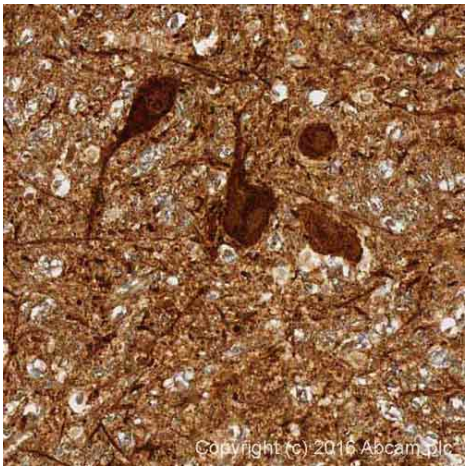
Blocking and diluting buffer and concentration: 5% NFDM/TBST



Flow Cytometry (Intracellular) - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

Intracellular Flow Cytometry analysis of 2% paraformaldehyde fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling alpha Synuclein with purified ab138501 at 1/200 dilution. The secondary antibody was Goat anti rabbit IgG (FITC) at 1/150 dilution.

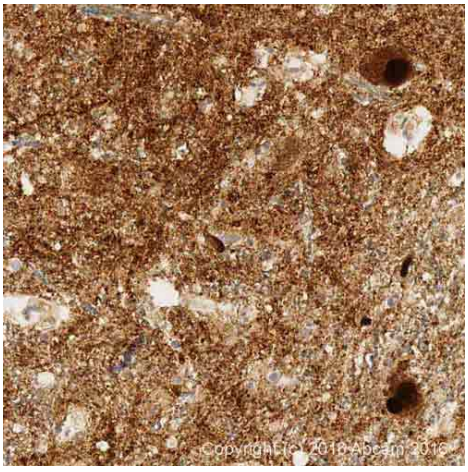
The Isotype control is Rabbit monoclonal IgG (green line).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

IHC image of alpha Synuclein staining in Normal human Substantia Nigra formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab138501, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



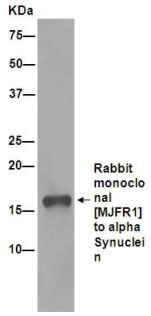
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

IHC image of alpha Synuclein staining in Parkinson Human Substantia Nigra formalin fixed paraffin embedded tissue section*, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab138501, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

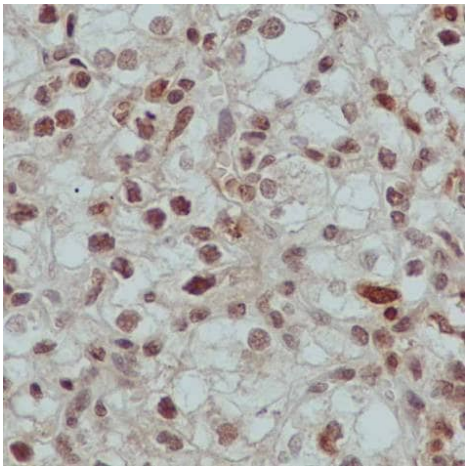
*Tissue obtained from the Human Research Tissue Bank,

supported by the NIHR Cambridge Biomedical Research Centre



alpha Synuclein was immunoprecipitated from Human fetal brain tissue using purified ab138501 at 1/600 dilution. Western blot was performed from the immunoprecipitate using purified ab138501. Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated was used as secondary antibody at 1/1000 dilution. Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Immunoprecipitation - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human clear cell carcinoma of kidney labeling alpha Synuclein with purified ab138501 at 1/150 dilution. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. Prediluted HRP Polymer for Rabbit IgG was used as the secondary antibody. Counter stained with Hematoxylin.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

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