

Product datasheet

Anti-Alpha-synuclein antibody [EPR20535] ab212184

KO VALIDATED Recombinant RabMAB

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Overview

Product name	Anti-Alpha-synuclein antibody [EPR20535]
Description	Rabbit monoclonal [EPR20535] to Alpha-synuclein
Host species	Rabbit
Tested applications	Suitable for: IHC-P, WB, IHC-Fr, IP
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant full length protein within Human Alpha-synuclein aa 1 to the C-terminus. The exact sequence is proprietary. Database link: P37840
Positive control	WB: Human Alpha-synuclein recombinant protein; Human cerebellum and brain lysates; Mouse and rat brain lysates. IHC-P: Human cerebral cortex and glioma tissues; Mouse and rat cerebral cortex tissues. IHC-Fr: Mouse hippocampus tissue. IP: Mouse brain lysate.
General notes	Our RabMAB [®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab[®] patents . This product is a recombinant rabbit monoclonal antibody .

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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20535
Isotype	IgG

Applications

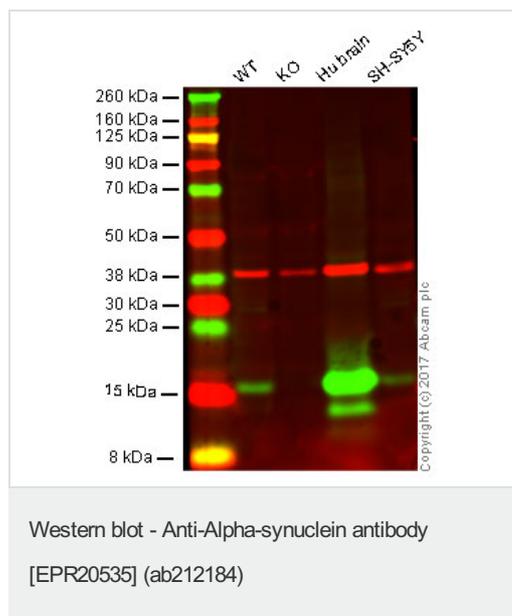
Our [Abpromise guarantee](#) covers the use of **ab212184** in the following tested applications.

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

Application	Abreviews	Notes
IHC-P		1/16000. 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 18 kDa (predicted molecular weight: 14 kDa).
IHC-Fr		1/100. Antigen retrieval: Heated citrate solution (10mM citrate pH 6.0 + 0.05% Tween-20).
IP		1/30.

Target

Function	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.
Tissue specificity	Expressed principally in brain but is also expressed in low concentrations in all tissues examined except in liver. Concentrated in presynaptic nerve terminals.
Involvement in disease	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
Sequence similarities	Belongs to the synuclein family.
Domain	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.
Post-translational modifications	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.
Cellular localization	Cytoplasm, cytosol. Membrane. Nucleus. Cell junction, synapse. Secreted. Membrane-bound in dopaminergic neurons.



Lane 1: Wild type HAP1 whole cell lysate (20 µg)

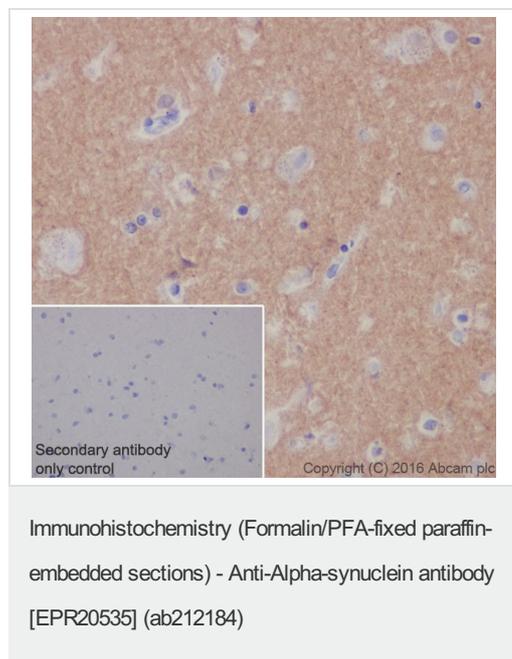
Lane 2: SNCA (alpha Synuclein) knockout HAP1 whole cell lysate (20 µg)

Lane 3: Human brain whole tissue lysate (20 µg)

Lane 4: SH-SY5Y whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab212184 observed at 14 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab212184 was shown to specifically react with SNCA (alpha Synuclein) in wild type cells as signal was lost in SNCA (alpha Synuclein) knockout cells. Wild-type and SNCA (alpha Synuclein) knockout samples were subjected to SDS-PAGE. ab212184 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at a 1/1000 dilution and 1/20000 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 1/20000 dilution for 1 hour at room temperature before imaging.

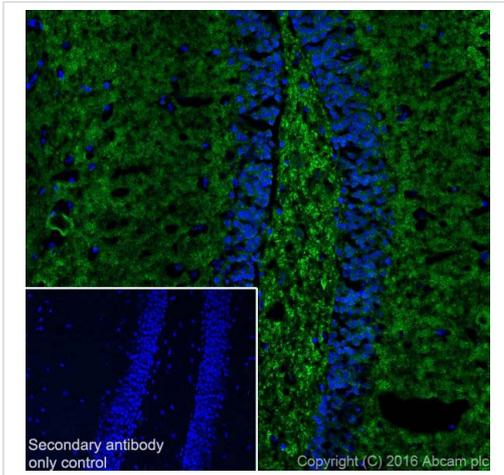


Immunohistochemical analysis of paraffin-embedded human cerebral cortex tissue labeling Alpha-synuclein with ab212184 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic staining on human cerebral cortex [PMID: 22112368]. 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.



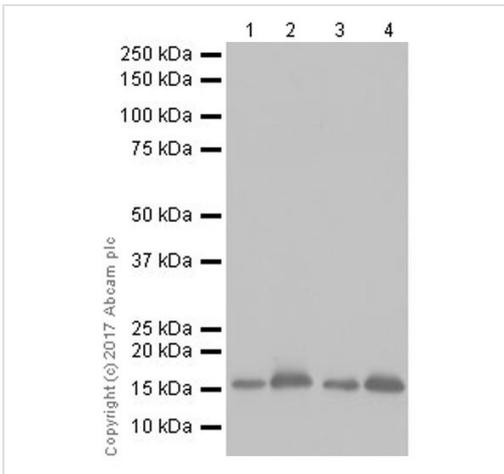
Immunohistochemistry (Frozen sections) - Anti-Alpha-synuclein antibody [EPR20535] (ab212184)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse hippocampus tissue labeling Alpha-synuclein with ab212184 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Cytoplasmic staining on mouse hippocampus (PMID: 22112368).

The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.



Western blot - Anti-Alpha-synuclein antibody [EPR20535] (ab212184)

All lanes : Anti-Alpha-synuclein antibody [EPR20535] (ab212184) at 1/1000 dilution

Lane 1 : Human cerebellum lysate

Lane 2 : Human brain lysate

Lane 3 : Mouse brain lysate

Lane 4 : Rat brain 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: 14 kDa

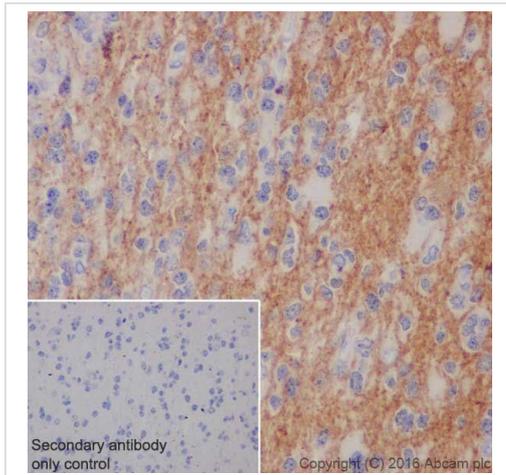
Observed band size: 18 kDa

[why is the actual band size different from the predicted?](#)

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFD/MBST.

The observed molecular weight is consistent with the literature (PMID: 11739566).



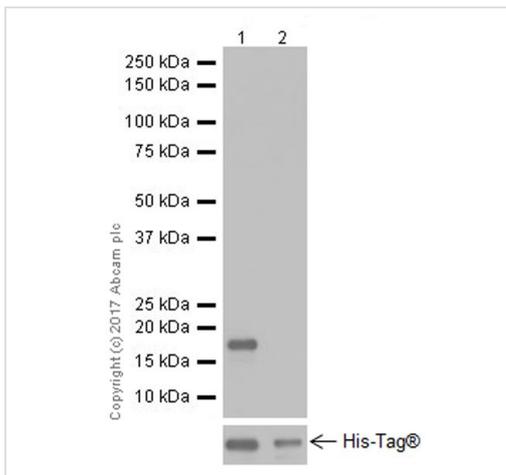
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [EPR20535] (ab212184)

Immunohistochemical analysis of paraffin-embedded human glioma tissue labeling Alpha-synuclein with ab212184 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic staining on human glioma [PMID: 22112368].
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-Alpha-synuclein antibody [EPR20535] (ab212184)

All lanes : Anti-Alpha-synuclein antibody [EPR20535] (ab212184) at 1/1000 dilution

Lane 1 : Human Alpha-synuclein recombinant protein

Lane 2 : Human Beta-synuclein recombinant protein

Lysates/proteins at 0.01 µg per lane.

Secondary

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

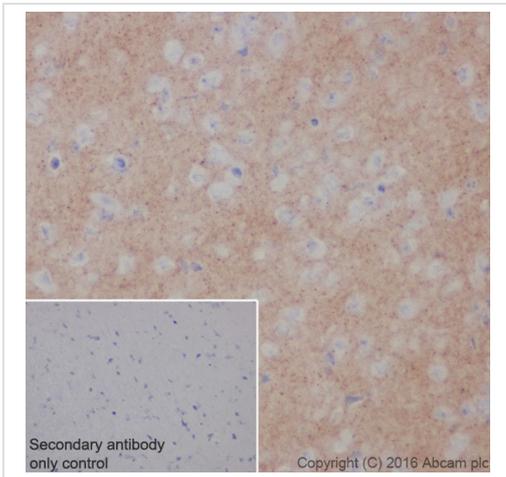
Predicted band size: 14 kDa

Observed band size: 18 kDa [why is the actual band size different from the predicted?](#)

Exposure time: 8 seconds

Blocking/Dilution buffer: 5% NFDm/TBST.

Human Alpha-synuclein recombinant protein contain aa1-140 with His-tag. Human Beta-synuclein recombinant protein contain aa1-134 with His-tag.



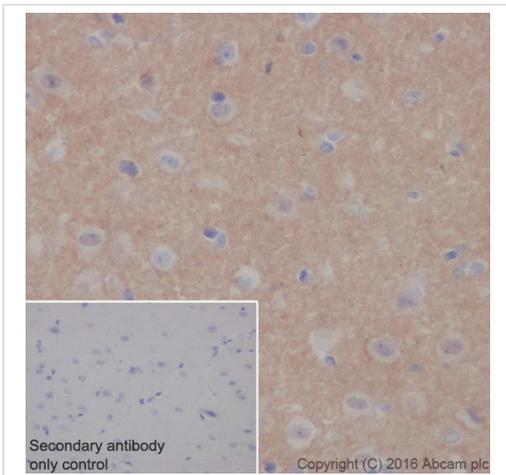
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [EPR20535] (ab212184)

Immunohistochemical analysis of paraffin-embedded mouse cerebral cortex tissue labeling Alpha-synuclein with ab212184 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic staining on mouse cerebral cortex [PMID: 22112368]. 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.



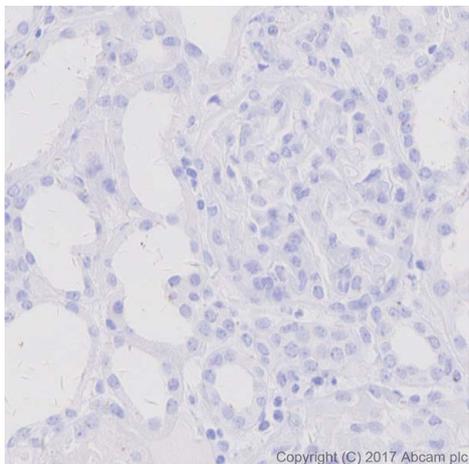
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [EPR20535] (ab212184)

Immunohistochemical analysis of paraffin-embedded rat cerebral cortex tissue labeling Alpha-synuclein with ab212184 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic staining on rat cerebral cortex [PMID: 22112368]. 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.



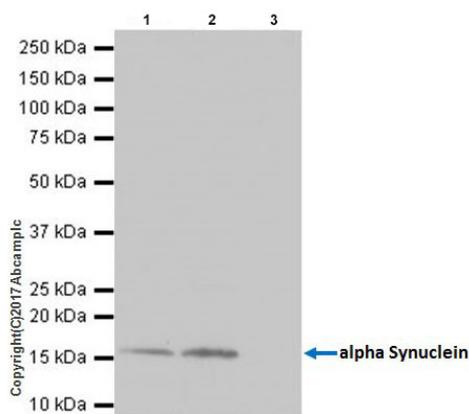
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [EPR20535] (ab212184)

Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling Alpha-synuclein with ab212184 at 1/16000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Negative control: No staining on human kidney. [PMID: 14997013].

Counter stained with Hematoxylin.

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



Immunoprecipitation - Anti-Alpha-synuclein antibody [EPR20535] (ab212184)

Alpha-synuclein was immunoprecipitated from 0.35 mg of mouse brain lysate with ab212184 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab212184 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution

Lane 1: Mouse brainlysate 10ug (Input).

Lane 2: ab212184 IP in mouse brain lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab212184 in mouse brain lysate.

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

Exposure time: 1 second.

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