abcam

Product datasheet

Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - BSA and Azide free ab214033

Recombinant RabMAb

10 Images

Overview

Product name	Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - BSA and Azide free
Description	Rabbit monoclonal [MJFR-14-6-4-2] to Alpha-synuclein aggregate - BSA and Azide free
Host species	Rabbit
Specificity	This antibody mainly recognizes alpha synuclein Filament, but also has weak cross-reactivity with alpha synuclein Monomer.
Tested applications	**Suitable for:** Dot blot, ICC/IF, IHC-P **Unsuitable for:** WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
Positive control	Dot Blot: Recombinant alpha-synuclein filament untreated and treated with 70% formic acid; Recombinant alpha-synuclein monomer. ICC/IF: Parkinson human substantia nigra, ReNcell VM (Human neural progenitor) cells. IHC-P: Rat dorsal root ganglian (DRG) tissue, human DLB brain tissue, mouse colon tissue sections.
General notes	ab214033 is the carrier-free version of **ab209538**. ab214033 is not suitable for WB or other denaturing conditions, as it is conformation-specific. This antibody is useful for studying Parkinson's disease and other synucleinopathies including dementia with Lewy bodies and multiple system atrophy. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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**.

This antibody was developed with support from The Michael J. Fox Foundation.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	MJFR-14-6-4-2
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab214033 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
Dot blot		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.

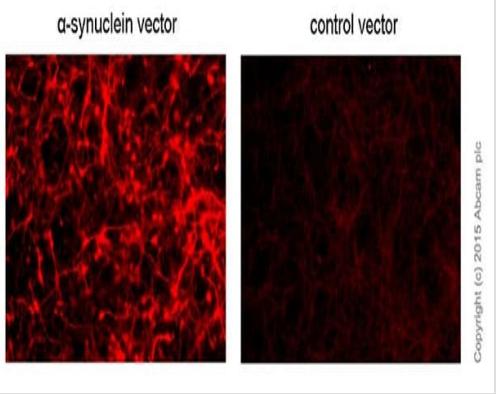
Application notes Is unsuitable for WB.

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.

Images



Immunocytochemistry/ Immunofluorescence - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - BSA and Azide free (ab214033)

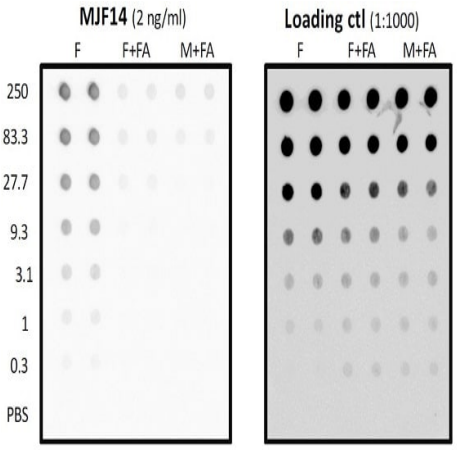
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized ReNcell VM (Human neural progenitor) cells labeling Alpha-synuclein aggregate with **ab209538** at 1/5000 dilution, followed by Alexa Fluor® 647 Donkey anti-Rabbit IgG (H+L) secondary antibody at 1/400 dilution (red).

Blocking buffer: 3% bovine serum albumin and 2% fetal bovine serum.

ReNcell VM cells were differentiated in media containing cAMP and GDNF (without bFGF or EGF) and transduced with Ad5C01 viral vector encoding human alpha-synuclein filament (Left image). Right image show control vector cells.

Images were reproduced courtesy of Charles River.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209538**).



Dot Blot - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - BSA and Azide free (ab214033)

Data is provided by Professor Poul Henning Jensen (Aarhus University Denmark).

Dot Blot showing the reactivity of **ab209538** (2 ng/ml) with

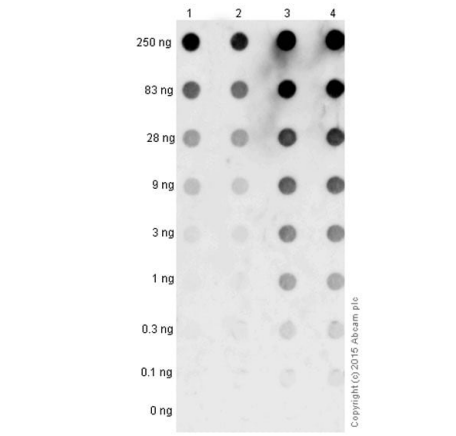
F: alpha synuclein Filament

F+FA: alpha synuclein Filament treated in 50% formic acid for 1 h 37oC prior to application to the dot blot.

M+FA: alpha synuclein Monomer treated in 50% formic acid for 1 h 37oC prior to application to the dot blot.

Loading control antibody (1:1000) reacts with Alpha-synuclein irrespectively of it being in a filament, oligomer or a monomer.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209538**).



Dot Blot - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - BSA and Azide free (ab214033)

Dot blot analysis of alpha-synuclein filament labeled with **ab209538** at 2.2 ng/ml.

Lane 1: Recombinant alpha-synuclein filament treated with 70% formic acid.

Lane 2: Recombinant alpha-synuclein filament treated with 70% formic acid.

Lane 3: Untreated recombinant alpha-synuclein filament.

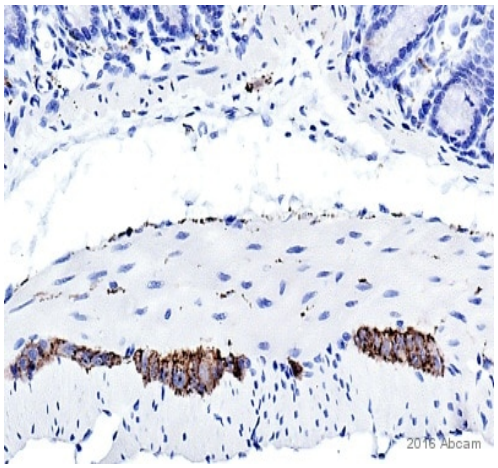
Lane 4: Untreated recombinant alpha-synuclein filament.

Alpha Synuclein filaments were generated using full length recombinant alpha-synuclein (aa1-140) by incubation in 20mM TRIS, pH 7.2 at 37 °C with agitation.

Denaturation of filaments with 70% formic acid reduces antibody recognition by 30-100 fold, demonstrating conformation specificity.

Data is provided by Professor Poul Henning Jensen, Aarhus University, Denmark.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209538**).

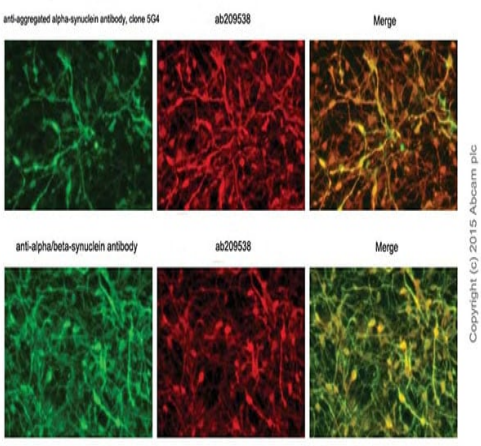


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - BSA and Azide free (ab214033)

This image is courtesy of an Abreview submitted by Carl Hobbs.

ab209538 staining Alpha-synuclein aggregate in mouse colon tissue sections by Immunohistochemistry (PFA perfusion fixed frozen sections). Tissue samples were fixed by perfusion with formaldehyde, cut into 20 micron slices, blocked with 2% BSA for 10 minutes at 21°C and antigen retrieval was by heat mediation in citrate buffer. The sample was incubated with primary antibody (1/2000 in TBS/BSA/azide) at 21°C for 2 hours. A biotin-conjugated goat anti-rabbit polyclonal (1/300) was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209538**).



Immunocytochemistry/ Immunofluorescence - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - BSA and Azide free (ab214033)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized ReNcell VM (Human neural progenitor) cells labeling Alpha-synuclein aggregate with **ab209538** at 1/5000 dilution, followed by Alexa Fluor® 647 Donkey anti-Rabbit IgG (H+L) secondary antibody at 1/400 dilution (red).

Upper panel counterstain: Anti-aggregated alpha-synuclein antibody clone 5G4 at 1/400 dilution, followed by AlexaFluor®488 secondary detection (green).

Lower panel counterstain: Anti-alpha/beta-synculein antibody at 1/200 dilution, followed by Alexa Fluor® 488 secondary detection (green).

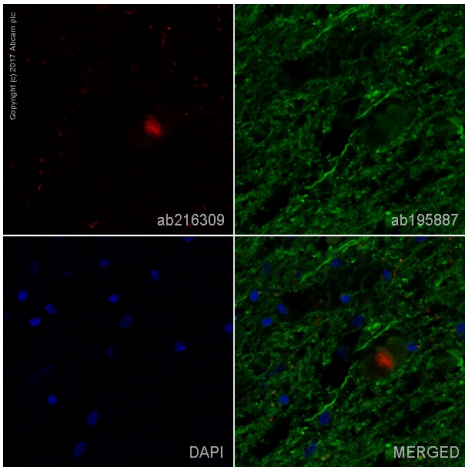
Blocking buffer: 3% bovine serum albumin and 2% fetal bovine serum.

ReNcell VM cells were differentiated in media containing cAMP and GDNF (without bFGF or EGF) and transduced with Ad5C01 viral vector encoding human WT alpha-synuclein filament.

Images were reproduced courtesy of Charles River.

This data was developed using the same antibody clone in a

different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209538**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - BSA and Azide free (ab214033)

Clone MJFR-14-6-4-2 (ab214033) has been successfully conjugated by Abcam. This image was generated using Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - Conformation-Specific (Alexa Fluor® 647). Please refer to **ab216309** for protocol details.

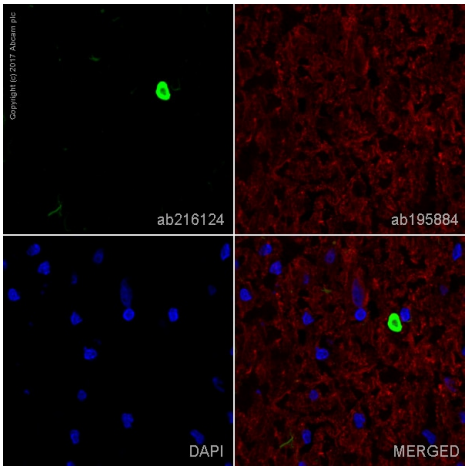
IHC image of alpha-synuclein aggregate staining in a section of formalin-fixed paraffin-embedded Parkinson human substantia nigra*.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) in a Biocare Medical NxGen pressure cooker using retrieval settings of 110^{O}C for 8 minutes. Non-specific protein-protein interactions were then blocked in TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 1% (w/v) BSA for 1h at room temperature. The section was then incubated overnight at +4°C in TBS containing 0.025% (v/v) Triton X-100 and 1% (w/v) BSA with **ab216309** at 1/5000 dilution (shown in red) and counterstained using **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

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

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre.



Immunohistochemistry (Frozen sections) - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - BSA and Azide free (ab214033)

Clone MJFR-14-6-4-2 (ab214033) has been successfully conjugated by Abcam. This image was generated using Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - Conformation-Specific (Alexa Fluor® 488). Please refer to **ab216124** for protocol details.

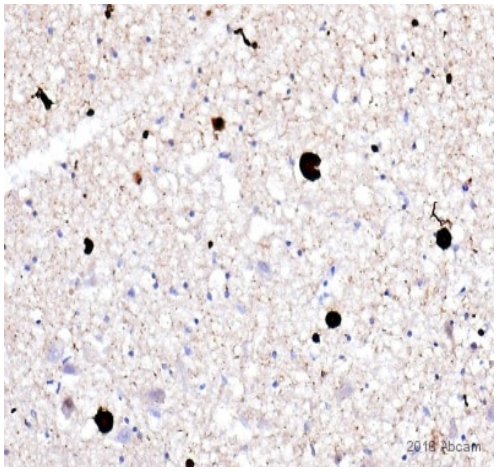
IHC image of alpha-synuclein aggregate staining in a section of frozen Parkinson human substantia nigra*.

The section was fixed using 10% formaldehyde in 1XPBS for 10 minutes. No antigen retrieval step was performed prior to staining. Non-specific protein-protein interactions were then blocked in TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 1% (w/v) BSA for 1h at room temperature. The section was then incubated overnight at +4°C in TBS containing 0.025% (v/v) Triton X-100 and 1% (w/v) BSA with **ab216124** at 1/100 dilution (shown in green) and counterstained using **ab195884**, Rat monoclonal to Tubulin (Alexa Fluor® 647), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

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

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre.

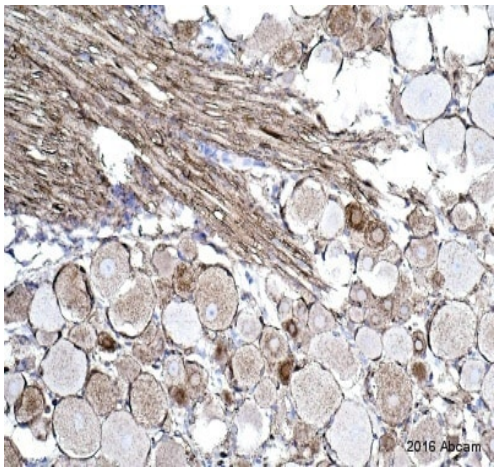


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - BSA and Azide free (ab214033)

This image is courtesy of an Abreview submitted by Carl Hobbs.

Ab209538 staining Alpha-synuclein aggregate in Human DLB brain tissue sections by Immunohistochemistry (Formalin/PFA fixed paraffin embedded sections). Tissue was fixed with paraformaldehyde and blocked with 2% BSA for 10 minutes at 21°C; antigen retrieval was by heat mediation in Citric acid. Samples were incubated with primary antibody (1/10,000 in TBS) for 2 hours at 21°C. A biotin conjugated anti-rabbit IgG Goat polyclonal was used as the secondary antibody at 1/300 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209538**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - BSA and Azide free (ab214033)

This IHC data was generated using the same anti-alpha synuclein aggregate antibody clone, MJFR-14-6-4-2, in a different buffer formulation (cat# **ab209538**).

ab209538 staining Alpha-synuclein aggregate in rat dorsal root ganglian (DRG) tissue sections by Immunohistochemistry (PFA perfusion fixed frozen sections). Tissue samples were fixed by perfusion with formaldehyde, cut into 20 micron slices and blocked with 2% BSA for 10 minutes at 21°C and antigen retrieval was by heat mediation in citrate buffer. The sample was incubated with primary antibody (1/2000 in TBS/BSA/azide) at 21°C for 2 hours. An Alexa Fluor® 555-conjugated Goat anti-rabbit polyclonal (1/300) was used as the secondary antibody.

Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - BSA and Azide free (ab214033)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors