# abcam

## Product datasheet

# Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] -Conformation-Specific ab209538

重组 RabMAb

★★★★★ 6 Abreviews 52 References 10 图像

概述

产品名称 Anti-Alpha-synuclein aggregate抗体[MJFR-14-6-4-2] - Conformation-Specific

描述 兔单克隆抗体[MJFR-14-6-4-2] to Alpha-synuclein aggregate - Conformation-Specific

宿主 Rabbit

特异性 This antibody mainly recognizes alpha synuclein Filament, but also has weak cross-reactivity with

alpha synuclein Monomer.

适用于: ICC/IF, Dot blot, IHC-P 经测试应用

不适用于: WB

种属反应性 与反应: Mouse, Rat, Human

免疫原 Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.

阳性对照 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.

常规说明 ab209538 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.

> 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.



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: 59% PBS, 40% Glycerol, 0.05% BSA

纯**度** Protein A purified

**克隆** 单克隆

**克隆编号** MJFR-14-6-4-2

**同种型** IgG

#### 应用

#### The Abpromise guarantee Abpromise™承诺保证使用ab209538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                                       |
|----------|------------------|--|
| ICC/IF   |                  | 1/5000.                                  |
| Dot blot |                  | Use a concentration of 0.0022 µg/ml.     |
| IHC-P    | <b>★★★★★ (4)</b> | Use at an assay dependent concentration. |

应用说明 ls unsuitable for WB.

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-

#### 翻译后修饰

terminus may regulate aggregation and determine the diameter of the filaments.

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.

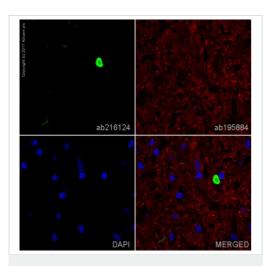
Ubiquitinated. The predominant conjugate is the diubiquitinated form.

Acetylation at Met-1 seems to be important for proper folding and native oligomeric structure.

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

图片

细胞定位



Immunocytochemistry/ Immunofluorescence - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2]

- Conformation-Specific (ab209538)

Clone MJFR-14-6-4-2 (<u>ab214033</u>) 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 <u>ab216124</u> for protocol details.

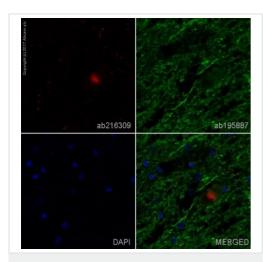
IHC image of alpha-synuclein aggregate staining in a section of frozen Parkinson human substantia nigra\*.

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 <u>ab216124</u> at 1/100 dilution (shown in green) and counterstained using <u>ab195884</u>, Rat monoclonal to Tubulin (Alexa Fluor<sup>®</sup> 647), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount<sup>®</sup>.

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.



Immunocytochemistry/ Immunofluorescence - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - Conformation-Specific (ab209538)

Clone MJFR-14-6-4-2 (<u>ab214033</u>) 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 <u>ab216309</u> for protocol details.

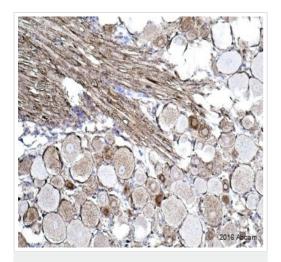
IHC image of alpha-synuclein aggregate staining in a section of formalin-fixed paraffin-embedded Parkinson human substantia nigra\*.

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°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 <a href="mailto:ab216309">ab216309</a> at 1/5000 dilution (shown in red) and counterstained using <a href="mailto:ab195887">ab195887</a>, Mouse monoclonal to alpha Tubulin (Alexa Fluor<sup>®</sup> 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount<sup>®</sup>.

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.

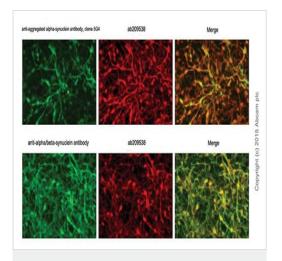
\*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - Conformation-Specific (ab209538)

This image is courtesy of an Abreview by Carl Hobbs.

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.



Immunocytochemistry/ Immunofluorescence - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - Conformation-Specific (ab209538)

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<sup>®</sup> 647 Donkey anti-Rabbit lgG (H+L) secondary antibody at 1/400 dilution (red).

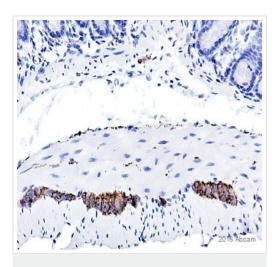
Upper panel counterstain: Anti-aggregated alpha-synuclein antibody clone 5G4 at 1/400 dilution, followed by AlexaFluor®488 secondary detection (green).

Lower panel counterstain: Anti-alpha/beta-synculein antibody at 1/200 dilution, followed by Alexa Fluor<sup>®</sup> 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.

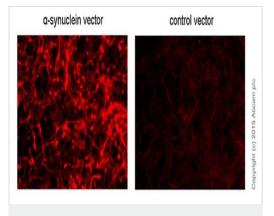
Images were reproduced courtesy of Charles River.



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.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - Conformation-Specific (ab209538)

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

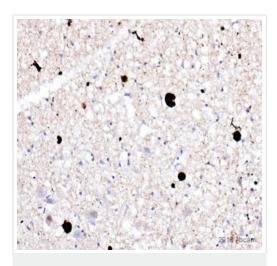


Immunocytochemistry/ Immunofluorescence - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - Conformation-Specific (ab209538) Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized ReNcell VM (Human neural progenitor) cells labeling Alpha-synuclein aggregate with ab209538 at 1/5000 dilution, followed by Alexa Fluor  $^{\circledR}647$  Donkey anti-Rabbit lgG (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.

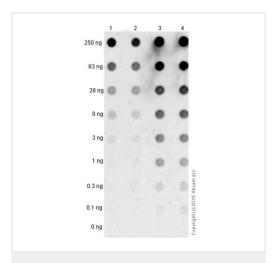
Images were reproduced courtesy of Charles River.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - Conformation-Specific (ab209538)

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.



Dot Blot - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - Conformation-Specific (ab209538)

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.

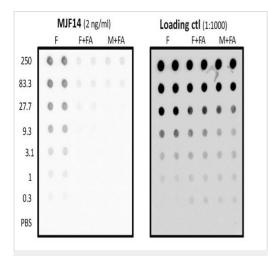
Lane 3: Untreated recombinant alpha-synuclein filament.

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.



Dot Blot - Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] - Conformation-Specific (ab209538)

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

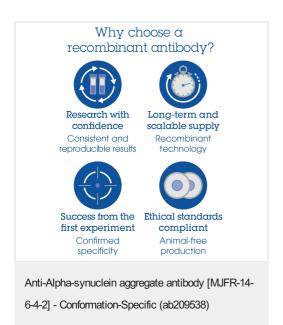
Dot Blot showing the reactivity of ab209538 (2 ng/ml) with

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.



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