### Overview

<table>
<thead>
<tr>
<th><strong>Product name</strong></th>
<th>Anti-Alpha-synuclein antibody [MJFR1]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Rabbit monoclonal [MJFR1] to Alpha-synuclein</td>
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<tr>
<td><strong>Host species</strong></td>
<td>Rabbit</td>
</tr>
<tr>
<td><strong>Tested applications</strong></td>
<td>Suitable for: WB, IHC-P, Flow Cyt, IP, ELISA, ICC/IF</td>
</tr>
<tr>
<td><strong>Species reactivity</strong></td>
<td>Reacts with: Human</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>Recombinant full length protein within Human Alpha-synuclein aa 1 to the C-terminus. The exact sequence is proprietary. Database link: P37840</td>
</tr>
<tr>
<td><strong>Epitope</strong></td>
<td>The epitope was mapped to amino acids 118-123 (VDPDNE).</td>
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<tr>
<td><strong>Positive control</strong></td>
<td>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: Hap1 cells.</td>
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<tr>
<td><strong>General notes</strong></td>
<td>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 fibrillar 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).</td>
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</tbody>
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Rat: We have preliminary internal testing data to indicate this antibody may not react with these
species. Please contact us for more information.

Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077).

See other anti-rabbit secondary antibodies that can be used with this antibody.

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.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

Properties

<table>
<thead>
<tr>
<th>Form</th>
<th>Liquid</th>
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<tbody>
<tr>
<td>Storage instructions</td>
<td>Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.</td>
</tr>
</tbody>
</table>
| Storage buffer | pH: 7.40
Preservative: 0.01% Sodium azide
 Constituents: 40% Glycerol, 0.05% BSA, 59% PBS |
| Purity        | Protein A purified |
| Clonality     | Monoclonal      |
| Clone number  | MJFR1           |
| Isotype       | IgG             |

Applications

Our Abpromise guarantee covers the use of ab138501 in the following tested applications.

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>WB</td>
<td>★★★★★</td>
<td>1/10000. Predicted molecular weight: 14 kDa. For unpurified use at 1/100000000 - 1/1000000000</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>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.</td>
</tr>
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<td>Application</td>
<td>Abreviews</td>
<td>Notes</td>
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</tbody>
</table>
| Flow Cyt    |           | 1/200. **For unpurified use at 1/20.**  
  *ab172730* - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. |
| IP          |           | 1/600. **For unpurified use at 1/50** |
| ELISA       |           | Use a concentration of 1 - 10 µg/ml. |
| ICC/IF      | ⭐⭐⭐⭐⭐    | 1/150. **For unpurified use at 1/15** |

### 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
Lane 1: Wild-type Hap1 cell lysate (20 µg)
Lane 2: SNCA knockout Hap1 cell lysate (20 µg)
Lane 3: Wild-type HEK-293T cell lysate (20 µg)
Lane 4: SNCA knockout HEK-293T cell lysate (20 µg)
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 react with Alpha-synuclein in wild-type HEK-293T cells. Loss of signal was observed when knockout sample ab263769 was used. Wild-type and Alpha-synuclein knockout samples were subjected to SDS-PAGE. ab138501 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 10000 dilution and 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.

Lane 1: Wild type HAP1 whole cell lysate (40 µg)
Lane 2: SNCA knockout HAP1 whole cell lysate (40 µg)
Lane 3: Human brain whole cell lysate (40 µg)
Lane 4: Mouse brain whole cell lysate (40 µg)
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 1/100000 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 1/10000 dilution for 1 hour at room temperature before imaging.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

Immunohistochemistry of 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 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).

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
Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

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

*why is the actual band size different from the predicted?*

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

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).

Immunocytochemistry/Immunofluorescence analysis of U87-MG (Human glioblastoma-astrocytoma epithelial cell line) cells labeling alpha Synuclein with unpurified ab138501 at 1/15 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).
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.

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.

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.
alpha Synuclein was immunoprecipitated from Human fetal brain tissue using unpurified ab138501 at 1/50 dilution. Western blot was performed from the immunoprecipitate using unpurified 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

Immohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human clear cell carcinoma of kidney labeling alpha Synuclein with unpurified ab138501 at 1/15 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) analysis of Human brain tissue labeling alpha Synuclein with unpurified ab138501 at 1/300 dilution.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Flow cytometry analysis of 2% paraformaldehyde fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling alpha Synuclein with unpurified ab138501 at 1/20 dilution. The secondary antibody was Goat anti rabbit IgG (FITC) at 1/150 dilution.

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

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