Product name: Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] ab51253

**Description**: Rabbit monoclonal [EP1536Y] to Alpha-synuclein (phospho S129)

**Host species**: Rabbit

**Specificity**: This antibody only detects alpha synuclein phosphorylated on Ser129. IHC-P: This antibody showed no staining in human hippocampus normal brain and showed staining in Parkinson's brain as expected.

**Tested applications**: Suitable for: IHC-FrFl, WB, Dot blot, ELISA, IHC-P, ICC/IF

**Unsuitable for**: Flow Cyt, IHC-Fr or IP

**Species reactivity**: Reacts with: Mouse, Rat, Human

**Predicted to work with**: Cow, Pig, Chimpanzee, Macaque monkey, Gorilla, Orangutan, Spider monkey

**Immunogen**: Synthetic peptide within Human Alpha-synuclein aa 100 to the C-terminus. The exact sequence is proprietary.

Database link: P37840

(Peptide available as ab188826)

**Positive control**: IHC-P: Human Parkinson Substantia Nigra tissue. WB: Sarkosyl-insoluble brain extract from mice transgenic for PrPA53T alpha-synuclein; Recombinant alpha-synuclein phosphorylated at S129; Rat and Mouse brain lysates.

**General notes**: 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.

**Properties**

- **7 Abreviews**
- **111 References**
- **9 Images**
Form  Liquid

Storage instructions  Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer  pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 59% PBS, 40% Glycerol, 0.5% BSA

Purity  Protein A purified

Clonality  Monoclonal

Clone number  EP1536Y

Isotype  IgG

Applications

Our Abpromise guarantee covers the use of ab51253 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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<tbody>
<tr>
<td>IHC-FrFl</td>
<td>1/5000.</td>
<td></td>
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<tr>
<td>WB</td>
<td>1/1000 - 1/5000. Detects a band of approximately 18 kDa (predicted molecular weight: 14 kDa). Can be blocked with Alpha-synuclein (phospho S129) peptide (ab188826). Good results have been obtained by treating the membrane with 0.4% PFA for 30 min at room temperature before blocking it with 5% milk.</td>
<td></td>
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<tr>
<td>Dot blot</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>ELISA</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-P</td>
<td>Use a concentration of 5 - 10 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
<td></td>
</tr>
<tr>
<td>ICC/IF</td>
<td>Use at an assay dependent concentration.</td>
<td></td>
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</table>

Application notes  Is unsuitable for Flow Cyt, IHC-Fr or IP.

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 promotes 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

Images

All lanes: Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] (ab51253) at 1/1000 dilution

Lane 1: Rat brain lysates, the membrane was incubated with alkaline phosphatase.

Lane 2: Rat brain lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/2000 dilution

Predicted band size: 14 kDa

Observed band size: 18 kDa

why is the actual band size different from the predicted?

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 20 seconds
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] (ab51253)

IHC image of alpha Synuclein (phospho S129) staining Human Parkinson Substantia Nigra tissue section*, previously antigen was retrieved by heat mediated with citrate buffer pH 6, fixed in formalin and embedded in paraffin. This section was incubated with ab51253 at 10 µg/mL for 15 mins at room temperature and detected using an HRP conjugated compact polymer system, performed on a Leica Bond™ system using the standard protocol F. DAB was used as the chromogen. Counterstained with haematoxylin and mounted with DPX. The inset negative control image is taken from an identical assay performed on Human normal Substantia Nigra.

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

Western blot - Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] (ab51253)

All lanes : Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] (ab51253) at 1/1000 dilution

Lane 1 : Mouse brain lysates, the membrane was incubated with alkaline phosphatase

Lane 2 : Mouse brain lysates

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/2000 dilution

Predicted band size: 14 kDa

Observed band size: 18 kDa why is the actual band size different from the predicted?

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 40 seconds
Expression of WT aSyn in the SN is toxic over time. Mouse brain sections immunostained for TH (red panels) and aSyn (using ab51253) (green panels) 1, 2 and 3 weeks after injection with vectors encoding for EGFP or WT aSyn. Scale bar for isolated channels 200 μm and for merged channels 100 μm.

All lanes: Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] (ab51253) at 1/1000 dilution

Lane 1: Sarkosyl-insoluble brain extract from mice transgenic for PrPA53T alpha-synuclein (line M83)

Lane 2: Recombinant alpha-synuclein phosphorylated at S129, 3ng

Predicted band size: 14 kDa

Direct ELISA antibody dose-response curve using ab51253. Antibody concentration of 0-5000 ng/mL. Antigen concentration of 1000 ng/mL. An alkaline phosphatase conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody.
Dot blot analysis of alpha Synuclein (pS129) peptide (Lane 1), alpha Synuclein (unmodified) peptide (Lane 2) labelling alpha Synuclein (pS129) with ab51253 at a dilution of 1/1000. Peroxidase conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody at a dilution of 1/2500.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

α-Synuclein (α-syn) and S129-phosphorylated α-synuclein protein levels in SNCA/SNCA mouse brains after 12 days of treatment with 4mM ambroxol (Amb). (A) Western blotting for α-synuclein (using ab1903) and serine 129 (S129)-phosphorylated α-synuclein protein (using ab51253) in the brainstem (example blots shown).

IHC image of alpha Synuclein (phospho S129) staining in free floating tgM83+/− or tgM83+ mouse brain tissue. The section was incubated with ab51253, 1/5000, for 15 hours at 4°C and detected using a Biotinylated conjugated Anti-Rabbit monocloal antibody, 1/200.

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