Antibody Information:

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

**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: WB, Dot blot, ELISA, IHC-P, ICC/IF. Unsuitable for: Flow Cyt, IHC-Fr or IP.

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

**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.

**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. 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. This product is a recombinant rabbit monoclonal antibody.
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>
</tr>
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<tbody>
<tr>
<td>WB</td>
<td>✭✭✭✭✭</td>
<td>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>
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<tr>
<td>Dot blot</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-P</td>
<td></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>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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</tbody>
</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 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


Images

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
IHC image of alpha Synuclein (phospho S129) staining in Human Parkinson 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 ab51253, 10µg/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. 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

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.

**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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