


Product datasheet

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

Recombinant RabMAb

★★★★★ [7 Abreviews](#) [293 References](#) [12 Images](#)

Overview

Product name	Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y]
Description	Rabbit monoclonal [EP1536Y] to Alpha-synuclein (phospho S129)
Host species	Rabbit
Specificity	<p>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.</p> <p>Mouse and rat species are recommended based on WB results, we do not guarantee IHC-FrFI and IHC-P for Mouse and rat.</p>
Tested applications	<p>Suitable for: IHC-FrFI, WB, Dot blot, ELISA, IHC-P</p> <p>Unsuitable for: Flow Cyt, IHC-Fr or IP</p>
Species reactivity	<p>Reacts with: Mouse, Rat, Human</p> <p>Predicted to work with: Cow, Pig, Chimpanzee, Macaque monkey, Gorilla, Orangutan, Spider monkey </p>
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (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. Mouse brain with Alzheimer's disease tissue lysate with and without alkaline phosphatase incubation. IHC-FI: Mouse brain tissue. ELISA: Alpha-synuclein (pS129) phospho peptide. Dot Blot: Human Alpha-synuclein (pS129) peptide.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

Properties

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 (glycerin, glycerine), 0.5% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1536Y
Isotype	IgG

Applications

The Abpromise guarantee 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.

Application	Abreviews	Notes
IHC-FrFI	★★★★★ (1)	Use at an assay dependent concentration.
WB	★★★★★ (4)	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.
Dot blot		Use at an assay dependent concentration.
ELISA		Use at an assay dependent concentration.
IHC-P		Use a concentration of 5 - 10 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

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.

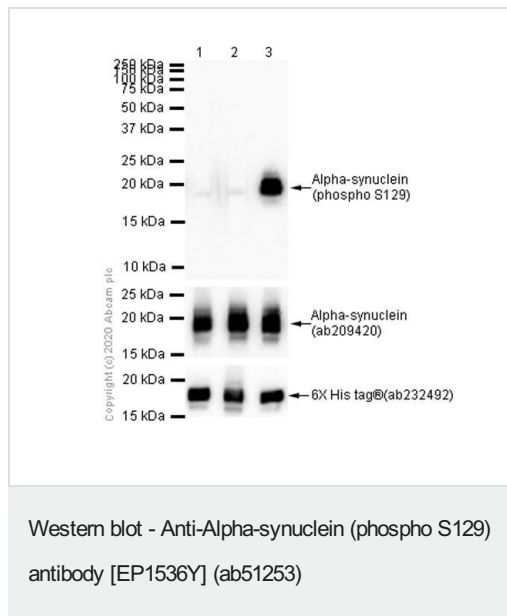
Ubiquitinated. The predominant conjugate is the diubiquitinated form.

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

Cellular localization

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

Images



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

Lane 1 : In vitro kinase assay of Alpha Synuclein phosphorylation using His tagged human full length recombinant alpha-synuclein protein in the presence of PLK2 (Polo-like kinase 2) but absence of ATP

Lane 2 : In vitro kinase assay of Alpha Synuclein phosphorylation using His tagged human full length recombinant alpha-synuclein protein in the presence of ATP but absence of PLK2 (Polo-like kinase 2)

Lane 3 : In vitro kinase assay of Alpha Synuclein phosphorylation using His tagged human full length recombinant alpha-synuclein protein in the presence of PLK2 (Polo-like kinase 2) and ATP

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 14 kDa

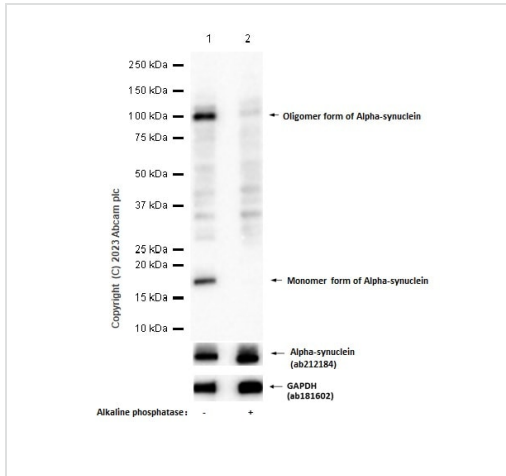
Observed band size: 17 kDa

Blocking and Diluting buffer and concentration - 5% NFDM/TBST

Exposure time: 3 seconds

Lysates used here were prepared from 1% SDS hot method.

Please refer to [here](#).



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 with Alzheimer's disease tissue lysate

Lane 2 : Mouse brain with Alzheimer's disease tissue lysate, membrane was incubated with alkaline phosphatase

Lysates/proteins at 20 μ g per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 14 kDa

Observed band size: 100,18 kDa

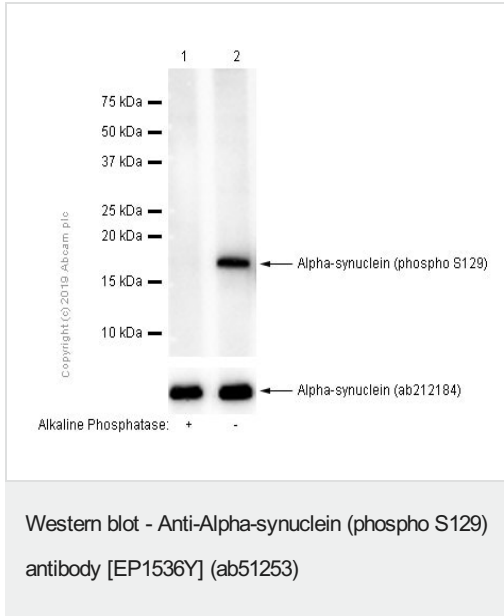
Exposure time: 140 seconds

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

This blot was developed using a higher sensitivity ECL substrate.

Band around 100kda corresponds to α S oligomer (PMID: 27637918, PMID: 12597857)

Lysates used here were prepared using RIPA method. We recommend 1% SDS hot lysate method to reduce the detection of oligomers. Please refer [here](#).



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

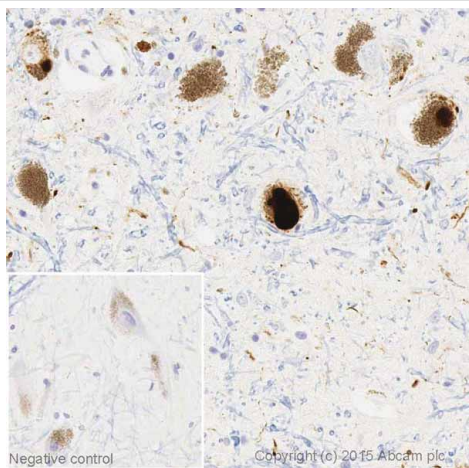
Observed band size: 18 kDa

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

Exposure time: 20 seconds

Lysates used here were prepared from 1% SDS hot method.

Please refer to [here](#).

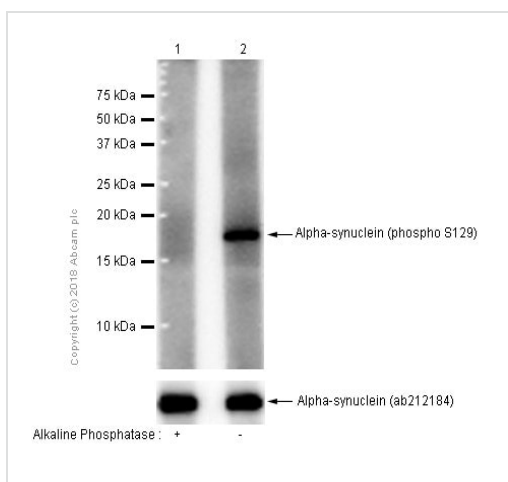


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] (ab51253)

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

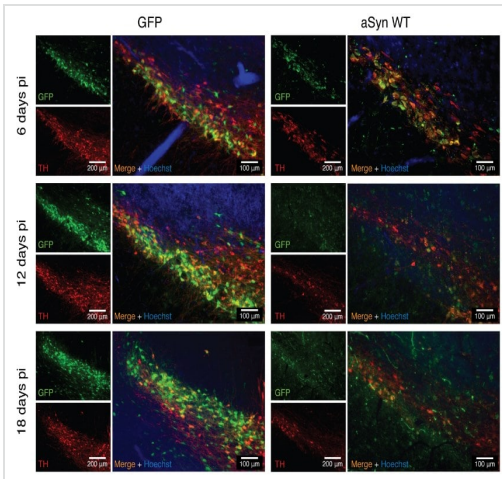
Observed band size: 18 kDa

Blocking/Diluting buffer and concentration: 5% NFD/MBST.

Exposure time: 40 seconds

Lysates used here were prepared from 1% SDS hot method.

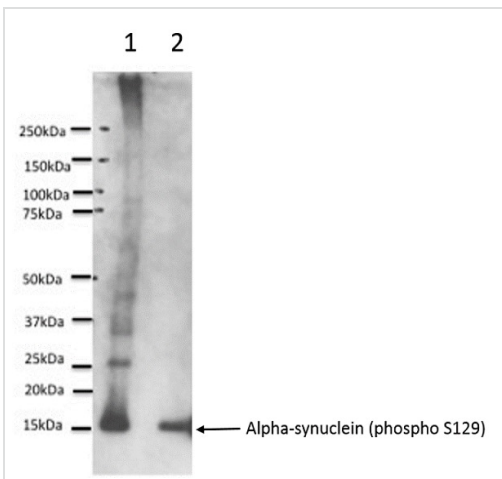
Please refer to [here](#).



Immunohistochemistry - Free Floating - Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] (ab51253)

This image is taken from Machado de Oliveira, R et al. PLoS Biol. 2017 Mar; 15(3): e2000374. S7a doi: 10.1371/journal.pbio.2000374 Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>.

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.



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

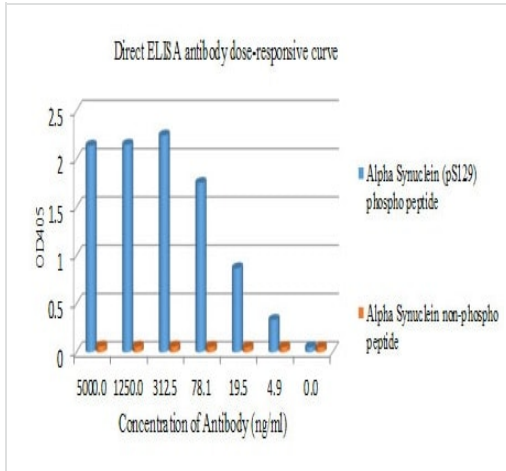
This image is courtesy of Professor Michael Goedert's team (Sophie Morgan and Masami Masuda-Suzukake).

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



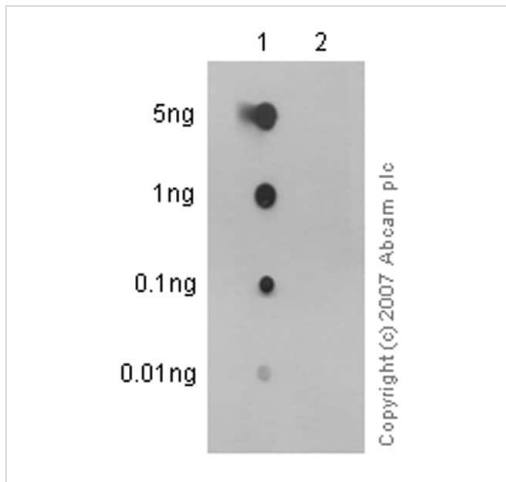
ELISA - Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] (ab51253)

Direct ELISA antibody dose-response curve using ab51253.

Antibody concentration of 0-5000 ng/mL.

Antigen (Alpha-synuclein (pS129) phospho peptide and Alpha-synuclein non-phospho peptide) concentration of 1000 ng/mL.

An alkaline phosphatase conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody.



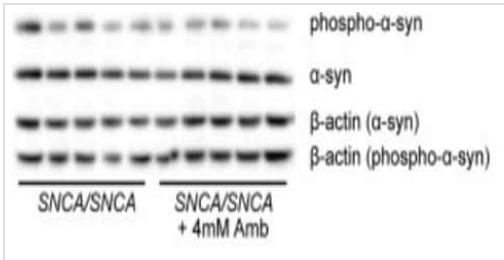
Dot Blot - Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] (ab51253)

Dot blot analysis of human alpha Synuclein (pS129) peptide (Lane 1), human 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.

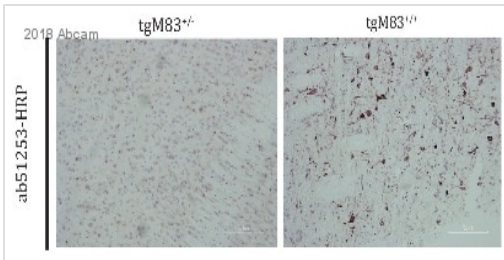
Exposure time: 3 minutes.



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

This image is taken from Mgdalska-Richards A et al. Ann Neurol. 2016 Nov; 80(5): 766–775. Fig 5 doi: 10.1002/ana.24790

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).



Immunohistochemistry - Free Floating - Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] (ab51253)

This image is courtesy of an abreview by Sophie Morgan

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 monoclonal antibody, 1/200.

Why choose α recombinant antibody?

- Research with confidence**
Consistent and reproducible results
- Long-term and scalable supply**
Recombinant technology
- Success from the first experiment**
Confirmed specificity
- Ethical standards compliant**
Animal-free production

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

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