# abcam

# Product datasheet

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

重组 RabMAb

★★★★★ 7 Abreviews 293 References 12 图像

概述

产品名称 Anti-Alpha-synuclein (phospho S129)抗体[EP1536Y]

描述 兔单克隆抗体[EP1536Y] to Alpha-synuclein (phospho S129)

**宿主** Rabbit

特异性 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.

Mouse and rat species are recommended based on WB results, we do not guarantee IHC-FrFI

and IHC-P for Mouse and rat.

经测试应用 适用于: IHC-FrFI, WB, Dot blot, ELISA, IHC-P

不适用于: Flow Cyt,IHC-Fr or IP

种属反应性 与反应: Mouse, Rat, Human

预测可用于: Cow, Pig, Chimpanzee, Macaque monkey, Gorilla, Orangutan, Spider monkey

A

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

(Peptide available as ab188826)

阳性对照 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.

常规说明 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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

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#### 性能

形式 Liquid

**存放说明** Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

**存储溶液** pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

纯**度** Protein A purified

**克隆** 单克隆 **克隆编号** EP1536Y

同种型 lgG

#### 应用

# The Abpromise guarantee Abpromise™承诺保证使用ab51253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
IHC-FrFI	**** (1)	Use at an assay dependent concentration.
WB	<b>★★★★★ (4)</b>	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.

应**用说明** Is unsuitable for Flow Cyt,IHC-Fr or IP.

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

翻译后修饰

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.

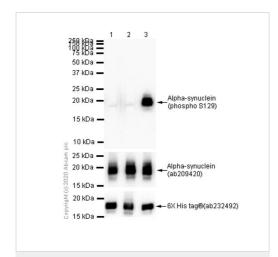
Acetylation at Met-1 seems to be important for proper folding and native oligomeric structure.

细胞定位

 $\hbox{\it Cytoplasm, cytosol. Membrane. Nucleus. Cell junction, synapse. Secreted. Membrane-bound in the property of the property$ 

dopaminergic neurons.

### 图片



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 :** 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 lgG 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.

**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 µg per lane.

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

# Secondary

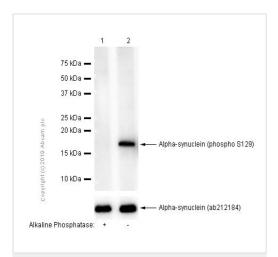
**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/20000 dilution

**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  $\alpha$ 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**.



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

Lane 2: Rat brain lysates

Lysates/proteins at 15 µg per lane.

# Secondary

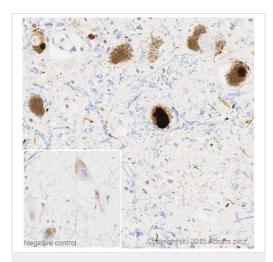
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/2000 dilution

**Predicted band size:** 14 kDa **Observed band size:** 18 kDa

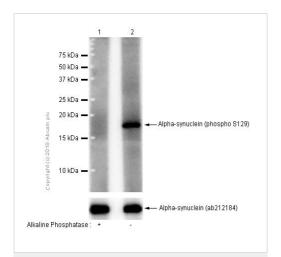
Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 20 seconds

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



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



Western blot - 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

**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

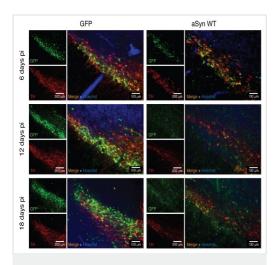
**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/2000 dilution

**Predicted band size:** 14 kDa **Observed band size:** 18 kDa

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

Exposure time: 40 seconds

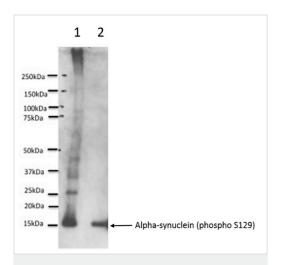
Lysates used here were prepared from 1% SDS hot method. Please refer to here.



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  $\mu m$  and for merged channels 100  $\mu m$ .

Immunohistochemistry - Free Floating - Anti-Alphasynuclein (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/.



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

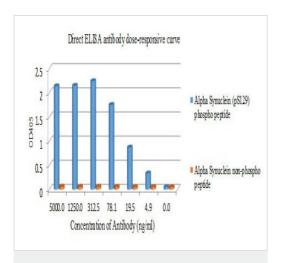
This image is courtesy of Professor Mchael Goedert's team (Sophie Morgan and Masami Masuda-Suzukake).

**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



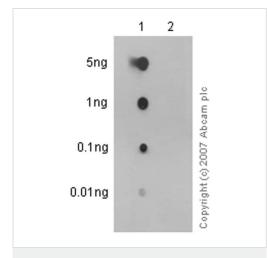
ELISA - Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] (ab51253)

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 lgG (1/1000) was used as the secondary antibody.

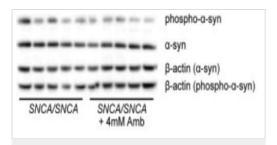


Dot Blot - Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] (ab51253)

Dot blot analysis of human alpha Synuclein (pS129) peptide (Lane 1), huamn alpha Synuclein (unmodified) peptide (Lane 2) labelling alpha Synuclein (pS129) with ab51253 at a dilution of 1/1000. Peroxidase conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody at a dilution of 1/2500.

Blocking and dilution buffer: 5% NFDM/TBST.

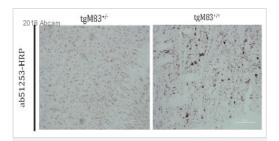
Exposure time: 3 minutes.



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

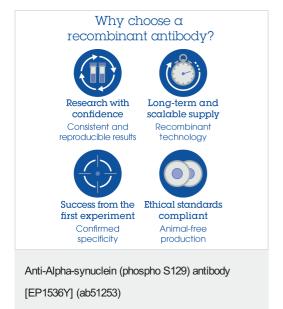
 $\alpha\text{-Synuclein}$  ( $\alpha\text{-syn}$ ) and S129-phosphorylated  $\alpha\text{-synuclein}$  protein levels in SNCA/SNCA mouse brains after 12 days of treatment with 4mM ambroxol (Amb). (A) Western blotting for  $\alpha\text{-synuclein}$  (using  $\underline{ab1903}$ ) and serine 129 (S129)-phosphorylated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



IHC image of alpha Synuclein (phospho S129) staining in free floating tgM83<sup>+/-</sup> or tgM83<sup>+/+</sup> 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.

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

This image is courtesy of an abreview by Sophie Morgan



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