# abcam

# Product datasheet

# Anti-alpha + beta Synuclein antibody [EP1646Y] ab51252



★★★★★★ <u>1 Abreviews</u> <u>24 References</u> 9 Images

#### Overview

Product name Anti-alpha + beta Synuclein antibody [EP1646Y]

**Description** Rabbit monoclonal [EP1646Y] to alpha + beta Synuclein

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, ICC/IF

Unsuitable for: IP

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

Immunogen Synthetic peptide within Human alpha + beta Synuclein aa 1-100 (N terminal). The exact

sequence is proprietary.

Positive control WB: Human fetal brain, human cerebellum, mouse brain and rat brain tissue lysates. ICC/IF:

PC12 and rat primary hippocampal neuron cells. Flow Cyt (intra): SH-SY5Y and U87-MG cells.

**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<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**.

#### **Properties**

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

1

Clonality Monoclonal
Clone number EP1646Y
Isotype IqG

#### **Applications**

#### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab51252 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
Flow Cyt (Intra)		1/30. For unpurified use at 1/1000.ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		1/5000. Detects a band of approximately 18 kDa (predicted molecular weight: 14 kDa). <b>For unpurified use at 1/50000.</b>
ICC/IF	<b>★★★★☆ (1)</b>	1/50 - 1/100.

**Application notes** 

Is unsuitable for IP.

#### **Target**

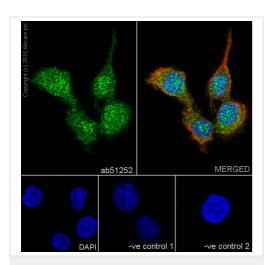
#### Relevance

Synucleins are small soluble proteins expressed primarily in neural tissues and in certain tumors. The family includes 3 known proteins, alpha synuclein, beta synuclein and gamma synuclein. All synucleins have in common a highly conserved alpha helical lipid binding motif with similarity to the class A2 lipid binding domains of the exchangeable apolipoproteins. The alpha and beta synuclein proteins are found primarily in brain tissue, where they are seen mainly in pre synaptic terminals. Alpha synuclein is believed to be a major component of Lewy bodies in Parkinson's disease. Mutations in alpha synuclein are associated with rare familial cases of early onset Parkinson's disease, Alzheimer's disease and several other neurodegenerative illnesses.

#### **Cellular localization**

Cytoplasmic. Membrane bound in dopaminergic neurons. Also found in the nucleus.

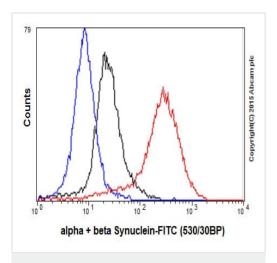
# **Images**



Immunocytochemistry/ Immunofluorescence - Antialpha + beta Synuclein antibody [EP1646Y] (ab51252)

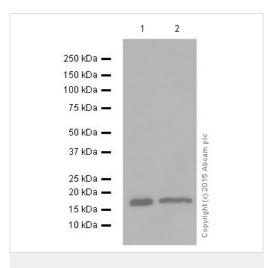
Immunocytochemistry/Immunofluorescence analysis of PC-12 cells labelling alpha + beta Synuclein with purified ab51252 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. <a href="mailto:ab150077">ab150077</a>, an Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit lgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. <a href="mailto:ab7291">ab7291</a>, a mouse anti-tubulin (1/1000) and <a href="mailto:ab150120">ab150120</a>, an Alexa Fluor<sup>®</sup> 594-conjugated goat anti-mouse lgG (1/1000). and secondary antibody, <a href="mailto:ab150120">ab150120</a>, an Alexa Fluor<sup>®</sup> 594-conjugated goat anti-mouse lgG (1/1000).

Control 2: <u>ab7291</u> (1/1000) and secondary antibody, <u>ab150077</u>, an Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit lgG (1/1000).



Flow Cytometry (Intracellular) - Anti-alpha + beta Synuclein antibody [EP1646Y] (ab51252)

Overlay histogram showing U87-MG cells fixed in 4% PFA and stained with purified ab51252 at a dilution of 1 in 30 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).



Western blot - Anti-alpha + beta Synuclein antibody [EP1646Y] (ab51252) **All lanes :** Anti-alpha + beta Synuclein antibody [EP1646Y] (ab51252) at 1/5000 dilution (purified)

Lane 1: Mouse brain tissue lysate

Lane 2: Rat brain tissue lysate

Lysates/proteins at 20 µg per lane.

# Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) ( $\underline{ab97051}$ ) at 1/20000

dilution

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

Blocking and dilution buffer: 5% NFDM /TBST.

250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —

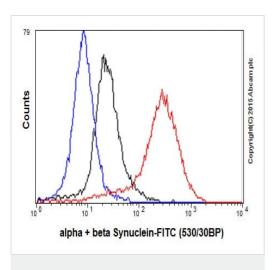
Western blot - Anti-alpha + beta Synuclein antibody [EP1646Y] (ab51252) Anti-alpha + beta Synuclein antibody [EP1646Y] (ab51252) at 1/5000 dilution (purified) + Human cerebellum tissue lysate at 20 µg

### **Secondary**

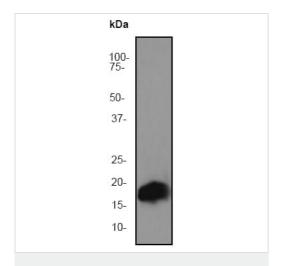
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

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

Blocking and dilution buffer: 5% NFDM /TBST.



Flow Cytometry (Intracellular) - Anti-alpha + beta Synuclein antibody [EP1646Y] (ab51252) Intracellular Flow Cytometry analysis of U87-MG cells labelling alpha + beta Synuclein with purified ab51252 at 1/30 (red). Cells were fixed with 4% paraformaldehyde. An Alexa Fluorr<sup>®</sup> 488-conjugated goat anti-rabbit lgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal lgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

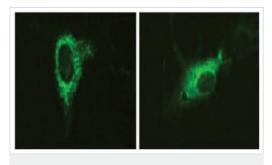


Western blot - Anti-alpha + beta Synuclein antibody [EP1646Y] (ab51252) Anti-alpha + beta Synuclein antibody [EP1646Y] (ab51252) at 1/50000 dilution (unpurified) + Human fetal brain tissue lysate at 10  $\mu g$ 

#### Secondary

HRP-conjugated goat anti-rabbit lgG at 1/2000 dilution

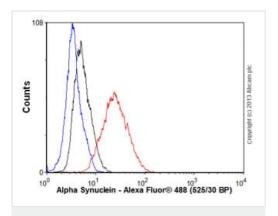
**Predicted band size:** 14 kDa **Observed band size:** 18 kDa



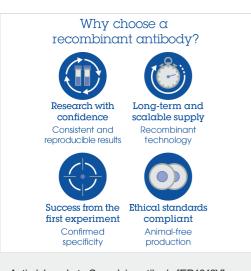
Immunocytochemistry/ Immunofluorescence - Antialpha + beta Synuclein antibody [EP1646Y] (ab51252)

Image from Hejjaoui Met al., J Am Chem Soc. 2012 Mar 21;134(11):5196-210. Epub 2012 Mar 13. Fig 4.; DOI: 10.1021/ja210866j; February 16, 2012, J. Am. Chem. Soc., 2012, 134 (11), pp 5196–5210, with permission from the American Chemical Society.

Immunocytochemistry/Immunofluorescence analysis of rat primary hippocampal neurons, staining alpha + beta Synuclein with unpurified ab51252. Cells were fixed, permeabilized, and blocked with 10% donkey serum at room temperature. Cells were incubated with primary antibody (1/1000) at 4°C for 24 hours. A Cy2-conjugated donkey anti-rabbit lgG was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-alpha + beta Synuclein antibody [EP1646Y] (ab51252) Overlay histogram showing SH-SY5Y cells stained with unpurifiedab51252 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (unpurified ab51252, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluorr 488 goat anti-rabbit lgG (H+L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (0.1 $\mu$ g/1x106 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Anti-alpha + beta Synuclein antibody [EP1646Y] (ab51252)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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