abcam

Product datasheet

Recombinant Human Alpha-synuclein protein aggregate (Active) ab218819

6 References 9 Images

Description

Product name	Recombinant Human Alpha-synuclein protein aggregate (Active)
Biological activity	Endogenous alpha-synuclein phosphorylation. 100 µM alpha synuclein protein monomer (**ab218818**) seeded with 10 µM alpha synuclein protein aggregate (ab218819) in 25 µM Thioflavin T (**ab120751**) (PBS pH 7.4, 100 µl reaction volume) generated a fluorescence intensity of 13,000 Relative Fluorescence Units after incubation at 37°C with shaking at 600 rpm for 24 hours. Fluorescence was measured by excitation at 450 nm and emission at 485 nm on a microplate reader. **Endotoxin Level:** 10-20 EU/mL
Purity	> 95 % SDS-PAGE. ab218819 was purified by ion-exchange.
Endotoxin level	<=20.000 Eu/ml
Expression system	Escherichia coli
Accession	**P37840**
Protein length	Full length protein
Animal free	No
Nature	Recombinant
Species	Human
Sequence	MDVFMKGLSK AKEGVVAAAE KTKQGVAEAA GKTKEGVLYV GSKTKEGVVH GVATVAEKTK EQVTNVGGAV VTGVTAVAQK TVEGAGSIAA ATGFVKKDQL GKNEEGAPQE GILEDMPVDP DNEAYEMPSE EGYQDYEPEA
Predicted molecular weight	14 kDa
Amino acids	1 to 140
Additional sequence information	(NP_000336.1) (GeneID 6622)
Description	Recombinant human Alpha-synuclein protein (Active)

Specifications

Our **Abpromise guarantee** covers the use of **ab218819** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Applications	Western blot Functional Studies SDS-PAGE
Form	Liquid
Additional notes	For best results, sonicate immediately prior to use. Sonication of PFFs is required in order to obtain fibrils with an average length of ~50nm, the optimal size for pathology.

Preparation and Storage

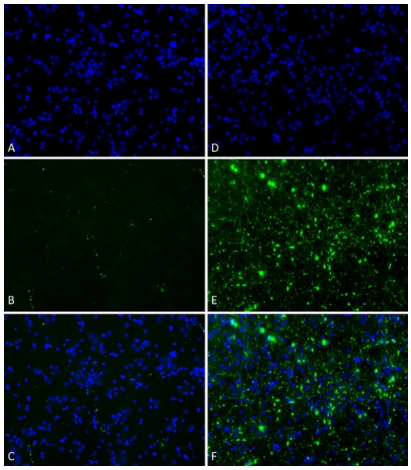
Stability and Storage	Shipped on Dry Ice. Store at -80°C. Avoid freeze / thaw cycle. Constituent: 95% PBS This product is an active protein and may elicit a biological response in vivo, handle with caution.

General Info

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

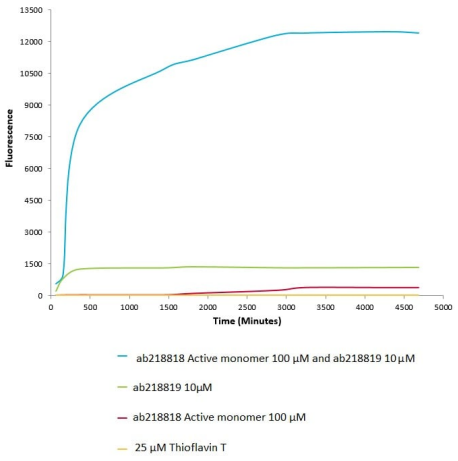
dopaminergic neurons.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Recombinant human Alpha-synuclein protein aggregate (Active) (ab218819)

Immunohistochemical analysis of primary rat hippocampal neurons showing lewy body inclusion formation when treated with active Alpha Synuclein Protein Aggregate (ab218819) at 4 µg/ml (D-F), but not when treated with control Alpha Synuclein Protein Aggregate (**ab218817**) at 4 µg/ml (A-C). Tissue: Primary hippocampal neurons. Species: Sprague-Dawley rat. Fixation: 4% formaldehyde from PFA. Primary antibody: Mouse anti-pSer129 Antibody at 1/1000 24 hours at 4°C. Secondary antibody: FITC Goat Anti-Mouse (green) at 1/700 for 1 hour at RT. Counterstain: Hoechst (blue) nuclear stain at 1/4000 for 1 hour at RT. Localization: Lewy body incluscions. Magnification: 20x.



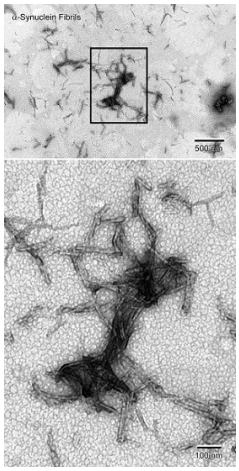
Functional Studies - Recombinant Human Alpha-synuclein protein aggregate (Active) (ab218819)

ab218819 seeds the formation of new alpha synuclein fibrils from the pool of alpha synuclein monomers.

Thioflavin T is a fluorescent dye that binds to beta sheet-rich structures, such as those in alpha synuclein fibrils. Upon binding, the emission spectrum of the dye experiences a red-shift, and increased fluorescence intensity.

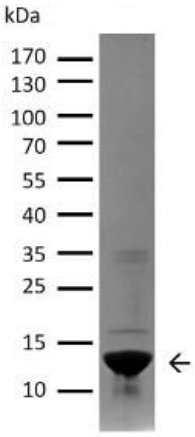
Thioflavin T emission curves show increased fluorescence (correlated to alpha synuclein protein aggregation) over time when 10 µM of ab218819 is combined with 100 µM of alpha synuclein monomer, as compared to ab218819 alone and alpha synuclein monomer alone.

Thioflavin T ex = 450 nm, em = 485 nm.



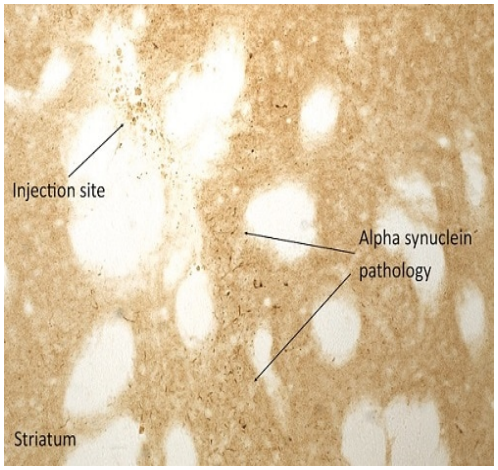
Electron Microscopy - Recombinant human Alpha-synuclein protein aggregate (Active) (ab218819)

TEM of active human alpha synuclein preformed fibrils (ab218819). Fibrils were sonicated and treated with uranyl acetate.



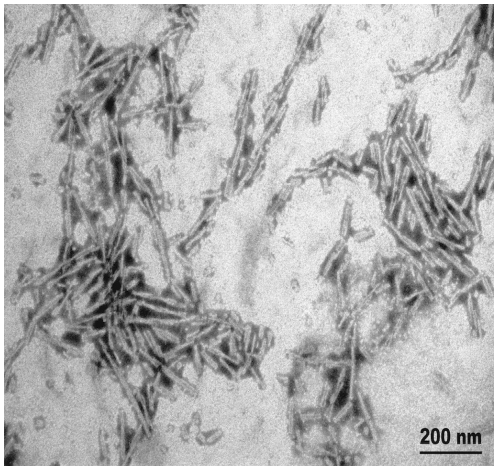
SDS-PAGE - Recombinant human Alpha-synuclein protein aggregate (Active) (ab218819)

SDS-PAGE analysis of ab218819.



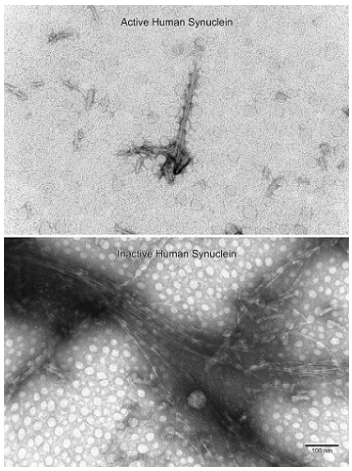
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Recombinant human Alpha-synuclein protein aggregate (Active) (ab218819)

Immunohistochemistry analysis of rat brain injected with active human alpha synuclein PFFs (ab218819). Species: Female Sprague-Dawley Rat. Rat was injected with 2µL active human alpha synuclein PFFs (ab218819) in each of 2 injection sites: AP+1.6, ML+2.4, DV-4.2 from skull; and AP-1.4, ML+0.2, DV-2.8 from skull. 30-days post-injection. Fixation: Saline perfusion followed by 4% PFA fixation for 48 hrs. Secondary Antibody: Biotin-SP Donkey Anti-Rabbit IgG (H+L) at 1:500 for 2 hours in cold room with shaking. ABC signal amplification, DAB staining. Alpha synuclein pathology is seen in the striatum close to an injection site.



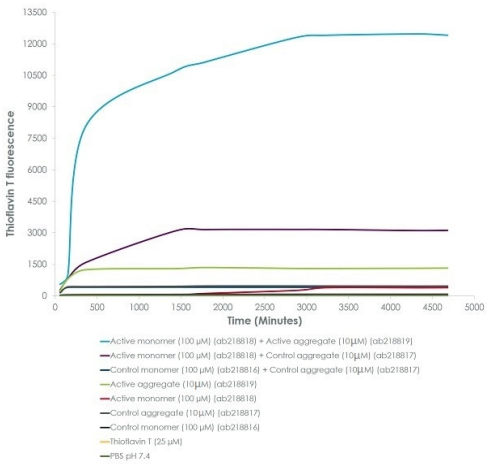
Electron Microscopy - Recombinant human Alpha-synuclein protein aggregate (Active) (ab218819)

TEM of ab218819.



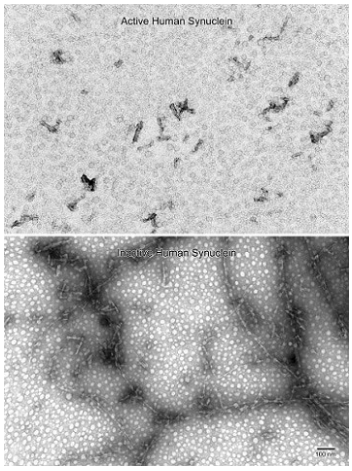
Electron Microscopy - Recombinant human Alpha-synuclein protein aggregate (Active) (ab218819)

TEM of active human alpha synuclein preformed fibrils (ab218819) (top) and control (inactive) human alpha synuclein preformed fibrils (**ab218817**) (bottom). Fibrils were sonicated and treated with uranyl acetate. The active fibrils are shorter than the inactive fibrils.



Functional Studies - Recombinant Human Alpha-synuclein protein aggregate (Active) (ab218819)

ThT emission curves show increased fluorescence (correlated to alpha-synucleinprotein aggregation) over time when 10 µM of active alpha-synuclein aggregate (ab218819) is combined with 100 µM of active alpha-synuclein monomer (**ab218818**) (light blue), as compared to when 100 µM of active alpha-synuclein monomer is combined with 10 µM of control alpha-synuclein aggregate (purple line), or 100 µM of control alpha-synuclein monomer (**ab218816**) is combined with 10 µM of control alpha-synuclein aggregate (**ab218817**) (dark blue). ThT ex = 450 nm, em = 485 nm. **View protocol**.



Electron Microscopy - Recombinant human Alpha-synuclein protein aggregate (Active) (ab218819)

TEM of active human alpha synuclein preformed fibrils (ab218819) (top) and control (inactive) human alpha synuclein preformed fibrils (**ab218817**) (bottom). Fibrils were sonicated and treated with uranyl acetate. The active fibrils are shorter than the inactive fibrils.

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