abcam

Product datasheet

Human SNCA (Alpha-synuclein) knockout HEK-293T cell lysate ab263769

2 Images

Overview

Product name	Human SNCA (Alpha-synuclein) knockout HEK-293T cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon2 and Insertion of the selection cassette in exon2.
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.

Tested applications **Suitable for:** WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab255537 - Human SNCA knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

Cell type epithelial

STR Analysis Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12

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.

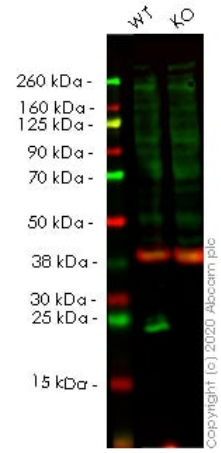
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab263769 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
WB		Use at an assay dependent concentration.

Images

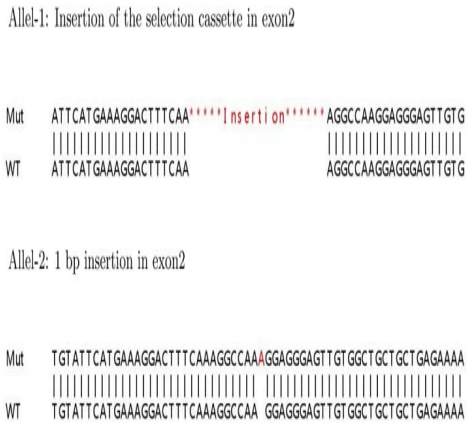


Western blot - Human SNCA knockout HEK293T cell lysate (ab263769)

Lane 1: Wild-type HEK-293T cell lysate (20ug)
Lane 2: SNCA knockout HEK-293T cell lysate (20ug)

Lanes 1- 2: Merged signal (red and green). Green - **ab138501** observed at 18 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab138501 Recombinant Anti-Alpha-synuclein antibody [MJFR1] was shown to specifically react with SNCA in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab255433** (knockout cell lysate ab263769) was used. Wild-type and SNCA knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab138501** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4^{o}C at 1 in 10000 and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Sanger Sequencing - Human SNCA knockout HEK293T cell lysate (ab263769)

Allele-1: Insertion of the selection cassette in exon2; Allele-2: 1 bp insertion in exon2

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