abcam

ab209420

Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free

Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420) is a rabbit recombinant monoclonal antibody provided in a PBS only buffer for easy conjugation. Suitable for **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF** in **Human**.

- KO validated for confirmed specificity
- BSA, sodium azide, and glycerol-free for easy conjugation
- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	MJFR1
Purification technique	Affinity purification Protein A
Epitope	The epitope was mapped to amino acids 118-123 (VDPDNE).
Concentration	1.021 - 1.054 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human

Dilution info	-
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target data

See full target information SNCA

Function	Neuronal protein that plays several roles in synaptic activity such as regulation of synaptic vesicle trafficking and subsequent neurotransmitter release (PubMed:20798282, PubMed:26442590, PubMed:28288128, PubMed:30404828). Participates as a monomer in synaptic vesicle exocytosis by enhancing vesicle priming, fusion and dilation of exocytotic fusion pores (PubMed:28288128, PubMed:30404828). Mechanistically, acts by increasing local Ca(2+) release from microdomains which is essential for the enhancement of ATP-induced exocytosis (PubMed:30404828). Also acts as a molecular chaperone in its multimeric membrane-bound state, assisting in the folding of synaptic fusion components called SNAREs (Soluble NSF Attachment Protein REceptors) at presynaptic plasma membrane in conjunction with cysteine string protein-alpha/DNAJC5 (PubMed:20798282). This chaperone activity is important to sustain normal SNARE-complex assembly during aging (PubMed:20798282). Also plays a role in the regulation of the dopamine neurotransmission by associating with the dopamine transporter (DAT1) and thereby modulating its activity (PubMed:26442590).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

What is this antibody validated in?
Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human samples.

What is the molecular weight of Alpha-synuclein?
Anti-Alpha-synuclein [MJFR1] - BSA and Azide free (ab209420) specifically detects a band for Alpha-synuclein (UniProt: P37840) at a molecular weight of 14kDa.

Specificity confirmed
The specificity of Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420) has been confirmed by Flow Cytometry testing in SNCA Knockout HEK-293 cell line, ab255433.

Other related products
We have a range of other formats of antibody clone [MJFR1] also available for your convenience: ab138501, Carrier free - ab209420, Carrier free - ab259612, ab309157, Carrier free - ab309158, Biotin - ab309388, HRP - ab314243, ab320839

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Species reactivity
Rat: We have preliminary internal testing data to indicate this antibody may not react with this species.
Please contact us for more information.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Collaborations
This antibody was developed with support from The Michael J. Fox Foundation.

THE MICHAEL J. FOX FOUNDATION
FOR PARKINSON'S RESEARCH

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary

Alpha-synuclein often referred to by alternate names such as SNCA is a protein of around 14 kDa mass. It mainly expresses in the brain particularly in presynaptic nerve terminals. This protein functions mechanically by stabilizing synaptic

	vesicles and maintaining synaptic function. It exists both in soluble monomer forms and as aggregates in protein filaments. Antibodies like 4D6 and EP1536Y target monomer forms of protein for more detailed studies.
Biological function summary	The alpha-synuclein protein plays critical roles in neuronal activity. It contributes to neurotransmitter release regulation by acting in the formation and plasticity of the presynaptic neuronal network. Alpha-synuclein doesn't usually form parts of large protein complexes but it may associate transiently with membranes and vesicular structures. The protein's monomer form has also been observed in alpha lines and related neuronal processes operating alongside various cellular functions.
Pathways	Synaptic vesicle trafficking and dopamine neurotransmitter release are significant areas involving the alpha-synuclein protein. In these pathways alpha-synuclein interacts with other proteins like synaptophysin and protein monomer monomerizations are intrinsic to these processes. Altered function or aggregation of alpha-synuclein disrupts these pathways influencing broader neurological functions.
Associated diseases and disorders	Alterations or accumulations of alpha-synuclein are strongly linked to Parkinson's disease and Lewy body dementia. In these conditions alpha-synuclein forms abnormal protein filaments known as Lewy bodies within neurons. These formations disrupt cellular processes and neuron health. Synucleinopathies such as these show connections with proteins like parkin and DJ-1 which also have key roles in these neurodegenerative diseases.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

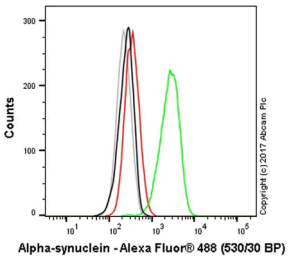
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

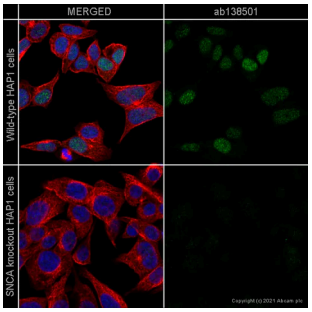
13 product images



Flow Cytometry (Intracellular) - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420)

Alpha-synuclein Flow Cytometry (Intracellular) staining using rabbit Anti-Alpha-synuclein antibody

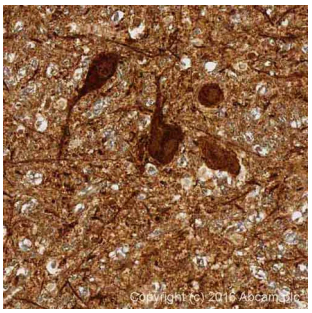
Overlay histogram showing HAP1 wildtype (green line) and HAP1-SNCA knockout cells (red line) stained with ab138501. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab138501, 1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) presorbed (ab150081) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG isotype control antibody (ab172730) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-SNCA knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab138501).



Immunocytochemistry/ Immunofluorescence - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420)

Alpha-synuclein Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Alpha-synuclein antibody

This data was developed using the same antibody clone in a different buffer formulation (ab138501). ab138501 staining Alpha-Synuclein in wild-type Hap1 cells (top panel) and SNCA knockout Hap1 cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab138501 at 1/10 000 dilution and ab7291 (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (ab150120) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



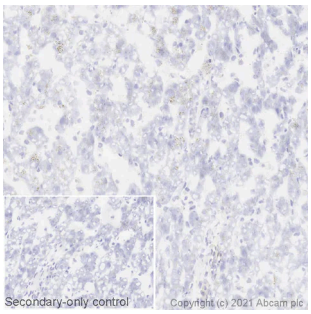
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420)

Alpha-synuclein Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Alpha-synuclein antibody

IHC image of alpha Synuclein staining in Normal human Substantia Nigra formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6,

epitope retrieval solution 1) for 20 mins. The section was then incubated with ab138501, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.
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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab138501).

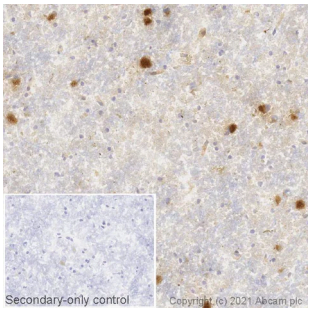


Immunohistochemistry (Frozen sections) - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420)

Alpha-synuclein Immunohistochemistry (Frozen sections) staining using rabbit Anti-Alpha-synuclein antibody

Negative control image: IHC image of Alpha synuclein staining in a section of frozen normal human liver* performed on a Leica BOND™ system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab209420, 0.5ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.
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



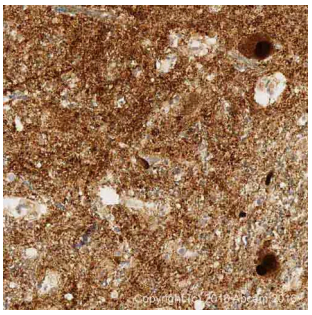
Immunohistochemistry (Frozen sections) - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420)

Alpha-synuclein Immunohistochemistry (Frozen sections) staining using rabbit Anti-Alpha-synuclein antibody

IHC image of Alpha synuclein staining in a section of frozen normal human Parkinson's brain* performed on a Leica BOND™ system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab209420, 0.5ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

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



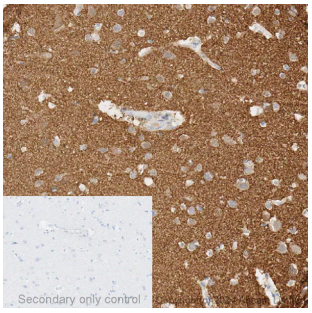
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420)

Alpha-synuclein Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Alpha-synuclein antibody

IHC image of alpha Synuclein staining in Parkinson Human Substantia Nigra formalin fixed paraffin embedded tissue section*, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab138501, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.
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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab138501).



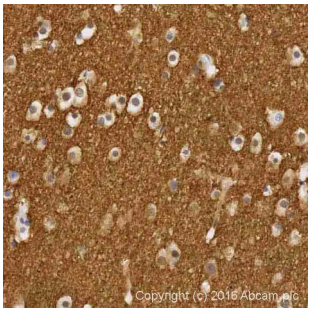
Immunohistochemistry - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab138501).

Immunohistochemical analysis of formalin fixed paraffin embedded human cerebrum labelling Alpha-synuclein with ab138501 at a concentration of 0.01 µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC2) 100°C, pH 8.5 for 32 mins.

ab138501 anti-Alpha-synuclein [MJFR1] was incubated for 16 mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual).



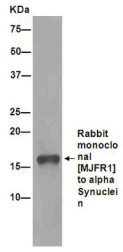
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420)

Alpha-synuclein Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Alpha-synuclein antibody

IHC image of alpha Synuclein staining in normal Human cerebral cortex formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6,

epitope retrieval solution 1) for 20 mins. The section was then incubated with ab138501, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.
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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab138501).



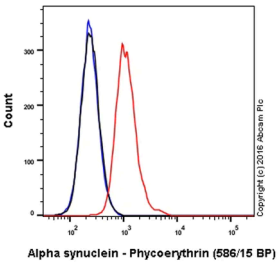
Immunoprecipitation - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420)

alpha Synuclein was immunoprecipitated from Human fetal brain tissue using purified ab138501 at 1/600 dilution. Western blot was performed from the immunoprecipitate using purified ab138501. Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated was used as secondary antibody at 1/1000 dilution. Blocking and dilution buffer and concentration: 5% NFDM/TBST.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab138501).

All lanes:
Immunoprecipitation - Anti-Alpha-synuclein antibody [MJFR1] (ab138501)

Predicted band size: 14 kDa

Observed band size: 18 kDa



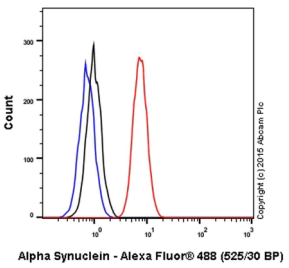
Flow Cytometry (Intracellular) - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420)

Alpha-synuclein Flow Cytometry (Intracellular) staining using rabbit Anti-Alpha-synuclein antibody

Clone MJFR1 (ab209420) has been successfully conjugated by Abcam. This image was generated using Anti-Alpha-synuclein antibody [MJFR1] (PE). Please refer to ab209306 for protocol details. Overlay histogram showing Neuro 2A (differentiated) cells stained with ab209306 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min at 22°C. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab209306, 1/2500 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was rabbit IgG (monoclonal) Phycoerythrin (ab209478) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Yellow/Green laser (561nm) and 586/15 bandpass filter.



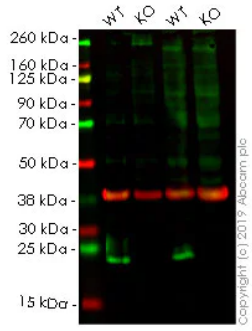
Flow Cytometry (Intracellular) - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420)

Alpha-synuclein Flow Cytometry (Intracellular) staining using rabbit Anti-Alpha-synuclein antibody

Clone MJFR1 (ab209420) has been successfully conjugated by Abcam. This image was generated using Anti-Alpha-synuclein antibody [MJFR1] (Alexa Fluor® 488). Please refer to ab195025 for protocol details.
Overlay histogram showing Neuro 2A cells stained with ab195025 (red line). The cells were fixed with 4% formaldehyde (10 min) then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. Cells were then incubated with the antibody (ab195025, 1/100 dilution) for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) Alexa Fluor® 488 (ab199091) used at the same concentration and conditions as the primary antibody. 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.

This antibody gave a positive signal in Neuro 2A cells fixed with 80% methanol (5 min) used under the same conditions.



Western blot - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420)

This data was developed using the same antibody clone in a different buffer formulation (ab138501).
Lanes 1 - 4: Merged signal (red and green). Green - ab138501 observed at 18 kDa. Red - loading control, ab8245 observed at 37 kDa.

ab138501 was shown to react with Alpha-synuclein in wild-type HEK-293T. Loss of signal was observed when knockout cell line ab255433 (knockout cell lysate ab263769) was used. Wild-type and Alpha-synuclein knockout samples were subjected to SDS-PAGE. ab138501 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 10000 dilution 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.

All lanes:
Western blot - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420) at 1/10000 dilution

Lane 1:
Wild-type HAP1 cell lysate at 20 µg

Lane 2:
SNCA knockout HAP1 cell lysate at 20 µg

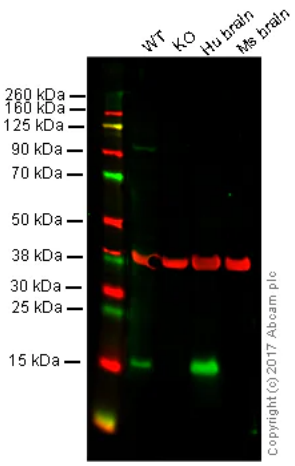
Lane 3:
Wild-type HEK-293T cell lysate at 20 µg

Lane 4:
SNCA knockout HEK-293T cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 14 kDa

Observed band size: 14 kDa



Western blot - Anti-Alpha-synuclein antibody [MJFR1] - BSA and Azide free (ab209420)

Lanes 1 - 4: Merged signal (red and green). Green - ab138501 observed at 14 kDa. Red - loading control, ab8245, observed at 37 kDa.
ab138501 was shown to specifically react with SNCA when SNCA knockout samples were used. Wild-type and SNCA knockout samples were subjected to SDS-PAGE. ab138501 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/10000 and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab138501).

All lanes:
Western blot - Anti-Alpha-synuclein antibody [MJFR1] (ab138501) at 1/10000 dilution

Lane 1:
Wild-type HAP1 whole cell lysate at 40 µg

Lane 2:
SNCA knockout HAP1 whole cell lysate at 40 µg

Lane 3:
Human brain whole cell lysate at 40 µg

Lane 4:
Mouse brain whole cell lysate at 40 µg

Predicted band size: 14 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.