abcam

Product datasheet

Anti-LRRK2 (phospho S935) antibody [UDD2 10(12)] ab133450

Recombinant RabMAb

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Overview

Product name	Anti-LRRK2 (phospho S935) antibody [UDD2 10(12)]
Description	Rabbit monoclonal [UDD2 10(12)] to LRRK2 (phospho S935)
Host species	Rabbit
Specificity	The antibody does not give a positive signal in U-87 MG, SH-SY-5Y and human fetal brain. Please contact our Scientific Support team if you have any question.
Tested applications	**Suitable for:** WB **Unsuitable for:** ICC/IF or IHC-P
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide within Human LRRK2 aa 900-1000 (phospho S935). The exact sequence is proprietary. Database link: **Q5S007** (Peptide available as **ab229694**)
Positive control	WB: GFP LRRK2, GFP LRRK2 S910A, GFP LRRK2 S935A, LRRK2 WT MEF, LRRK2 WT MEF, Lymphoblastoid and RAW 264.7 cell lysates.
General notes	This antibody was developed with the support of The Michael J. Fox Foundation (MJFF) and in partnership with Dr. Dario Alessi (MRC Protein Phosphorylation Unit, University of Dundee) to help accelerate LRRK2 research. Dr. Alessi has characterized several unique and high quality LRRK2 rabbit monoclonal antibodies, generated by Epitomics, to be made widely available for PD research community. LRRK2 (Leucine-rich repeat kinase 2, dardarin) is a multi-domain protein belonging to the ROCO family of proteins that contains a kinase and GTPase domain among its many protein interaction domains. LRRK2 is mutated in a significant number of Parkinson's disease (PD) patients. Mutations in this gene account for 4% of PD, and are observed in 1% of sporadic PD patients. The most common mutation replaces glycine 2019 with a serine that results in increased LRRK2 kinase activity. This indicates that inhibitors of LRRK2 kinase activity might be of therapeutic benefit for the treatment of Parkinson's disease and has stimulated much activity in this field of research. The Dundee-MJFF LRRK2 PhosphoSer935 antibody will be of great utility in further understanding the link between 14-3-3 binding to LRRK2 and Parkinson's disease as well as

assessing the efficacy of LRRK2 inhibitors that are being developed. It should be noted the Dundee-MJFF antibody is highly selective and sensitive and can readily be used to monitor LRRK2 Ser935 phosphorylation in immunoblot analysis of 2-20 microgram amounts of whole cell extract. The Dundee-MJFF LRRK2 PhosphoSer935 recognizes both mouse and human endogenous LRRK2.

A BSA and Azide free version of this product is available as **ab172382**.

Abcam recommended secondaries - Goat Anti-Rabbit HRP (**ab205718**) and Goat Anti-Rabbit Alexa Fluor® 488 (**ab150077**). Or search our wide range of secondary antibodies for use with your experiment.

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

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

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. Stable for 12 months at -20°C.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	UDD2 10(12)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab133450 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

Application	Abreviews	Notes
WB	★★★★★ (2)	1/1000. Detects a band of approximately 286 kDa (predicted molecular weight: 286 kDa).Can be blocked with **Human LRRK2 (phospho S935) peptide (ab229694)**.

Application notes Is unsuitable for ICC/IF or IHC-P.

Target

Function Positively regulates autophagy through a calcium-dependent activation of the CaMKK/AMPK signaling pathway. The process involves activation of nicotinic acid adenine dinucleotide phosphate (NAADP) receptors, increase in lysosomal pH, and calcium release from lysosomes. Together with RAB29, plays a role in the retrograde trafficking pathway for recycling proteins, such as mannose 6 phosphate receptor (M6PR), between lysosomes and the Golgi apparatus in a retromer-dependent manner. Regulates neuronal process morphology in the intact central nervous system (CNS). Plays a role in synaptic vesicle trafficking. Phosphorylates PRDX3. Has GTPase activity. May play a role in the phosphorylation of proteins central to Parkinson disease.

Tissue specificity Expressed in the brain. Expressed in pyramidal neurons in all cortical laminae of the visual cortex, in neurons of the substantia nigra pars compacta and caudate putamen (at protein level). Expressed throughout the adult brain, but at a lower level than in heart and liver. Also expressed in placenta, lung, skeletal muscle, kidney and pancreas. In the brain, expressed in the cerebellum, cerebral cortex, medulla, spinal cord occipital pole, frontal lobe, temporal lobe and putamen. Expression is particularly high in brain dopaminoceptive areas.

Involvement in disease Parkinson disease 8

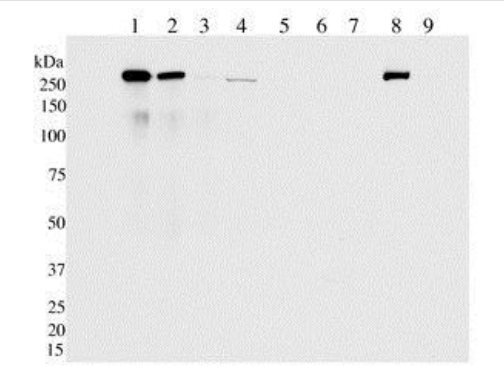
Sequence similarities Belongs to the protein kinase superfamily. TKL Ser/Thr protein kinase family.
Contains 12 LRR (leucine-rich) repeats.
Contains 1 protein kinase domain.
Contains 1 Roc domain.
Contains 7 WD repeats.

Domain The seven-bladed WD repeat region is critical for synaptic vesicle trafficking and mediates interaction with multiple vesicle-associated presynaptic proteins.
The Roc domain mediates homodimerization and regulates kinase activity.

Post-translational modifications Autophosphorylated.

Cellular localization Membrane. Cytoplasm. Perikaryon. Mitochondrion. Golgi apparatus. Cell projection, axon. Cell projection, dendrite. Endoplasmic reticulum. Cytoplasmic vesicle, secretory vesicle, synaptic vesicle membrane. Endosome. Lysosome. Mitochondrion outer membrane. Mitochondrion inner membrane. Mitochondrion matrix. Predominantly associated with intracytoplasmic vesicular and membranous structures (By similarity). Localized in the cytoplasm and associated with cellular membrane structures. Predominantly associated with the mitochondrial outer membrane of the mitochondria. Colocalized with RAB29 along tubular structures emerging from Golgi apparatus. Localizes in intracytoplasmic punctate structures of neuronal perikarya and dendritic and axonal processes.

Images



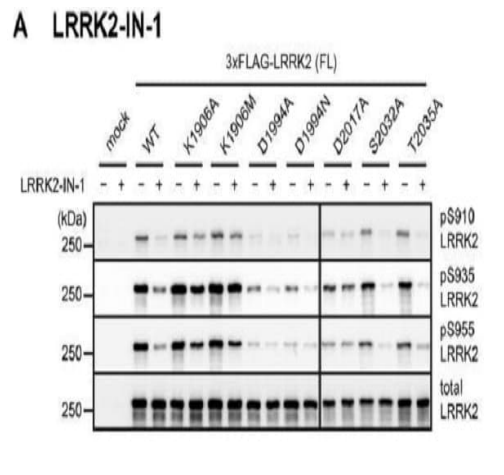
Western blot - Anti-LRRK2 (phospho S935) antibody [UDD2 10(12)] (ab133450)

All lanes : Anti-LRRK2 (phospho S935) antibody [UDD2 10(12)] (ab133450) at 1/1000 dilution (unpurified)

Lane 1 : GFP LRRK2 lysate at 5 µg
Lane 2 : GFP LRRK2 S910A lysate at 5 µg
Lane 3 : GFP LRRK2 S935A lysate at 5 µg
Lane 4 : LRRK2 WT MEF lysate at 20 µg
Lane 5 : LRRK2 WT MEF lysate from LRRK2 IN1 treated cells at 20 µg
Lane 6 : LRRK2 KO MEF lysate at 20 µg
Lane 7 : LRRK2 KO MEF lysate from LRRK2 IN1 treated cells at 20 µg
Lane 8 : Lymphoblastoid lysate at 30 µg
Lane 9 : Lymphoblastoid lysate from LRRK2 IN1 treated cells at 30 µg

Secondary
All lanes : Goat anti-rabbit HRP conjugated antibody at 1/2000 dilution

Predicted band size: 286 kDa



Western blot - Anti-LRRK2 (phospho S935) antibody [UDD2 10(12)] (ab133450)

Ito et al PLoS One. 2014 May 16;9(5):e97988. doi: 10.1371/journal.pone.0097988. eCollection 2014. Fig 3. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

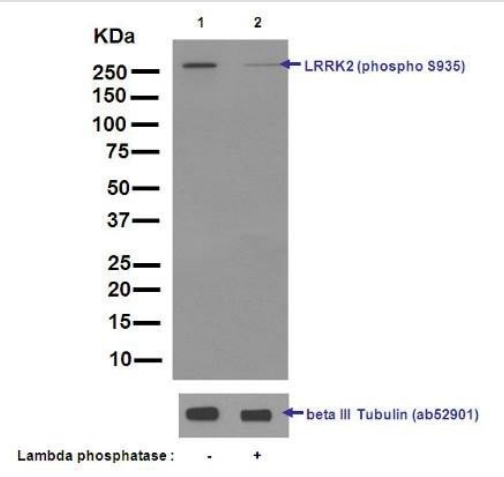
Inhibitor-induced dephosphorylation of kinase-inactive LRRK2.

HEK-293 cells transfected with wild-type, or mutated LRRK2 (K1906A, K1906M, D1994A, D1994N, D2017A, S2032A or T2035A) were treated with 3 µM LRRK2-IN-1 (LRRK2 inhibitor) for 30 min. The phosphorylation of LRRK2 at Ser910, Ser935, or Ser955 was examined by immunoblotting. This showed that the responses of LRRK2 to the inhibitor varied among mutants.

LRRK2 (phospho S935) was detected using **ab133449**.

LRRK2 (phospho S935) was detected using ab133450.

LRRK2 (phospho S955) was detected using **ab169521**.



Western blot - Anti-LRRK2 (phospho S935) antibody [UDD2 10(12)] (ab133450)

All lanes : Anti-LRRK2 (phospho S935) antibody [UDD2 10(12)] (ab133450) at 1/5000 dilution (purified)

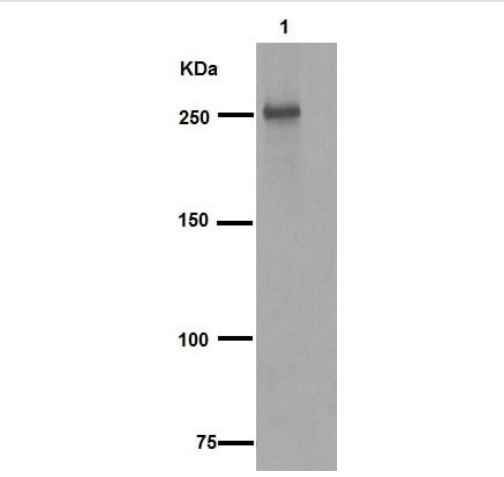
Lane 1 : WT-LRRK2 cell lysate - untreated
Lane 2 : WT-LRRK2 cell lysate - treated with Lambda phosphatase

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 286 kDa
Observed band size: 286 kDa

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



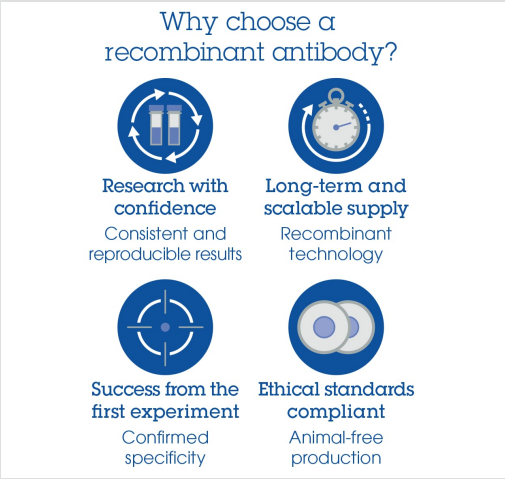
Western blot - Anti-LRRK2 (phospho S935) antibody [UDD2 10(12)] (ab133450)

Anti-LRRK2 (phospho S935) antibody [UDD2 10(12)] (ab133450) at 1/1000 dilution (purified) + RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cell lysate at 10 µg

Secondary
Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 286 kDa
Observed band size: 286 kDa

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



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