abcam

Product datasheet

Anti-PINK1 antibody [EPR20730] - BSA and Azide free ab232374

KO VALIDATED Recombinant RabMAb

4 Images

Overview

Product name	Anti-PINK1 antibody [EPR20730] - BSA and Azide free
Description	Rabbit monoclonal [EPR20730] to PINK1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	ICC/IF: HeLa cells treated with 10uM carbonyl cyanide 3-chlorophenylhydrazone (CCCP) for 24 hours. WB: HeLa cells (+/- treatment with 10uM carbonyl cyanide 3-chlorophenylhydrazone (CCCP, **ab141229**) for 24 hours) whole cell lysate; human PINK1 recombinant protein (aa156-507) .
General notes	ab232374 is the carrier-free version of **ab216144**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20730
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab232374 in the following tested applications.

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

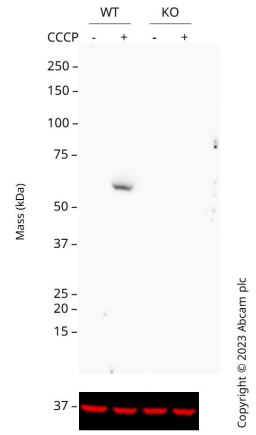
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 63 kDa.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

Target

Function	Protects against mitochondrial dysfunction during cellular stress, potentially by phosphorylating mitochondrial proteins. Involved in the clearance of damaged mitochondria via selective autophagy (mitophagy). It is necessary for PARK2 recruitement to dysfunctional mitochondria to initiate their degradation.
Tissue specificity	Highly expressed in heart, skeletal muscle and testis, and at lower levels in brain, placenta, liver, kidney, pancreas, prostate, ovary and small intestine. Present in the embryonic testis from an early stage of development.
Involvement in disease	Defects in PINK1 are the cause of Parkinson disease type 6 (PARK6) [MIM:605909]. A neurodegenerative disorder characterized by parkinsonian signs such as rigidity, resting tremor and bradykinesia. A subset of patients manifest additional symptoms including hyperreflexia, autonomic instability, dementia and psychiatric disturbances. Symptoms show diurnal fluctuation and can improve after sleep.
Sequence similarities	Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. Contains 1 protein kinase domain.

Post-translational modifications	Autophosphorylated.
Cellular localization	Mitochondrion outer membrane. Cytoplasm > cytosol.

Images



Western blot - Anti-PINK1 antibody [EPR20730] - BSA and Azide free (ab232374)

All lanes : Anti-PINK1 antibody [EPR20730] (**ab216144**) at 1/200 dilution

Lane 1 : Wild-type HEK-293 Vehicle Control CCCP, **ab141229** (0 µM, 24h) cell lysate
Lane 2 : Wild-type HEK-293 Treated CCCP, **ab141229** (10 µM, 24 h) cell lysate
Lane 3 : PINK1 knockout HEK-293 Vehicle Control CCCP, **ab141229** (0 µM, 24 h) cell lysate
Lane 4 : PINK1 knockout HEK-293 Treated CCCP, **ab141229** (10 µM, 24 h) cell lysate

Lysates/proteins at 20 µg per lane.

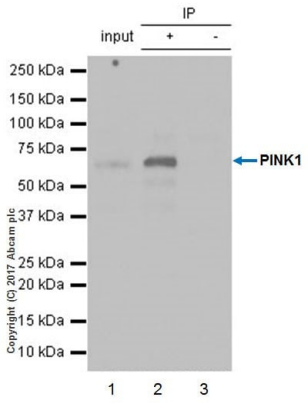
Performed under reducing conditions.

Predicted band size: 63 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab216144**).

Anti-PINK1 antibody [EPR20730] (**ab216144**) staining at 1/200 dilution shown in black; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. **ab216144** was shown to bind specifically to PINK1. A band was observed at 60 kDa in wild-type HEK-293 cell lysates with no signal observed at this size in PINK1 knockout cell line **ab266393** (knockout cell lysate **ab257030**). Membranes were blocked in 5 % milk in TBS-0.1 % Tween 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T and incubated with secondary antibodies for 1 h at room temperature, washed again four times before development with Optiblot (ECL reagent **ab133456**) and imaged with 20 minutes exposure time. Secondary antibodies used were HRP conjugated Goat anti-Rabbit (H+L) and Goat anti-Mouse IgG H&L 680RD at

1/20000 dilution.



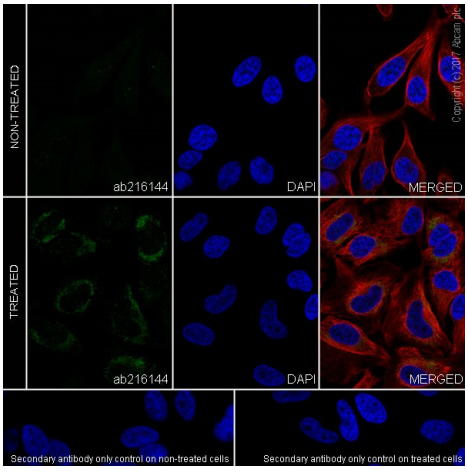
Immunoprecipitation - Anti-PINK1 antibody [EPR20730] - BSA and Azide free (ab232374)

PINK1 was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma)(treated with 10uM carbonyl cyanide 3-chlorophenylhydrazone (CCCP) for 24 hours) whole cell lysate with **ab216144** at 1/30 dilution. Western blot was performed from the immunoprecipitate using **ab216144** at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

Lane 1: HeLa (CCCP-treated) lysate 10 µg (Input).
Lane 2: **ab216144** IP in HeLa (CCCP-treated) lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab216144** in HeLa (CCCP-treated) whole cell lysate.

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 (**ab216144**).

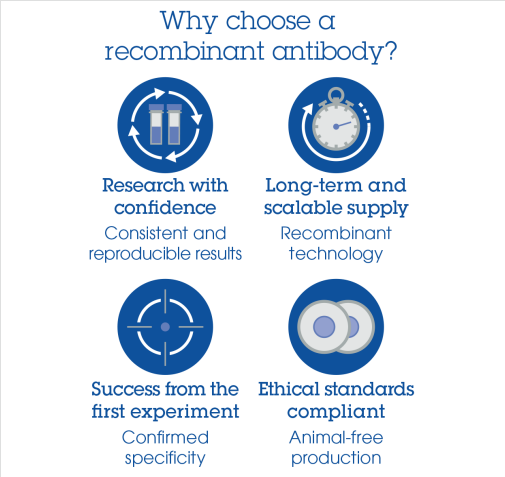


Immunocytochemistry/ Immunofluorescence - Anti-PINK1 antibody [EPR20730] - BSA and Azide free (ab232374)

Immunofluorescent analysis of 4 % paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma)(+/- treatment with 10µM carbonyl cyanide 3-chlorophenylhydrazone (CCCP) for 24 hours) cells labeling PINK1 with **ab216144** at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on HeLa cells treated with 10µM carbonyl cyanide 3-chlorophenylhydrazone (CCCP) for 24 hours. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) at 1/200 dilution (red).

The negative controls are as follows:
-ve control: PBS, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab216144**).



Anti-PINK1 antibody [EPR20730] - BSA and Azide free (ab232374)

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