abcam

Product datasheet

Anti-PINK1 antibody [EPR20730] ab216144

敲除验证 重组 RabMAb

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概述

产品名称	Anti-PINK1抗体[EPR20730]
描述	兔单克隆抗体[EPR20730] to PINK1
宿主	Rabbit
经测试应用	**适用于:** WB, ICC/IF, IP
种属反应性	**与反应:** Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HeLa cells (+/- treatment with 10uM carbonyl cyanide 3-chlorophenylhydrazone (CCCP, **ab141229**) for 24 hours) whole cell lysate; human PINK1 recombinant protein (aa156-507) . ICC/IF: HeLa cells treated with 10uM carbonyl cyanide 3-chlorophenylhydrazone (CCCP, **ab141229**) for 24 hours. IP: HeLA cells (treated with 10uM carbonyl cyanide 3-chlorophenylhydrazone (CCCP, **ab141229**) for 24 hours) whole cell lysate.
常规说明	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR20730
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab216144于以下的经测试应用

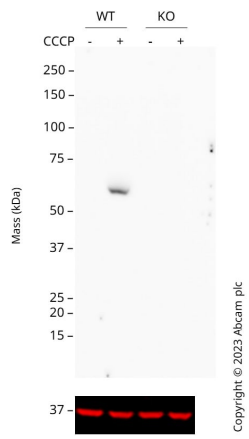
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★☆ (3)	1/1000. Predicted molecular weight: 63 kDa.
ICC/IF	★★★★☆ (1)	1/500.
IP		1/30.

靶标

功能	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.
组织特异性	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.
疾病相关	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.
序列相似性	Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. Contains 1 protein kinase domain.
翻译后修饰	Autophosphorylated.
细胞定位	Mitochondrion outer membrane. Cytoplasm > cytosol.

图片



Western blot - Anti-PINK1 antibody [EPR20730] (ab216144)

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

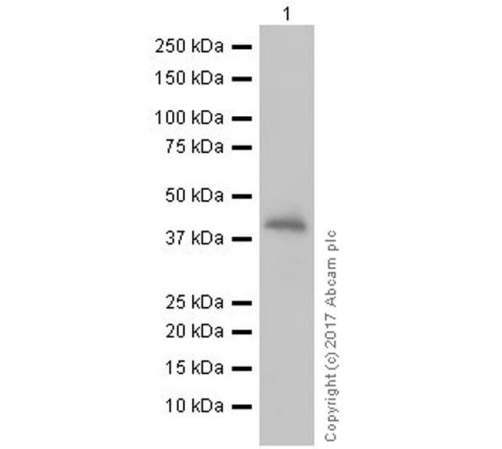
Lane 1 : Wild-type HEK-293 Vehicle Control CCCP, **ab141229** (0 µM, 24h) cell lysate
Lane 2 : Wild-type HEK-293 Treated CCCP, **ab141229** (10 µM, 24 h) cell lysate
Lane 3 : PINK1 knockout HEK-293 Vehicle Control CCCP, **ab141229** (0 µM, 24 h) cell lysate
Lane 4 : PINK1 knockout HEK-293 Treated CCCP,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

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 63 kDa

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.



Western blot - Anti-PINK1 antibody [EPR20730] (ab216144)

Anti-PINK1 antibody [EPR20730] (ab216144) at 1/1000 dilution + Human PINK1 recombinant protein (aa156-507), 10 ng

Secondary

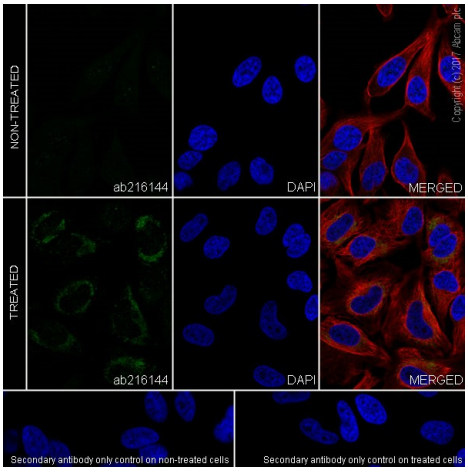
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution (Goat Anti-Rabbit IgG H&L (HRP))

Developed using the ECL technique.

Predicted band size: 63 kDa
Observed band size: 38 kDa

Exposure time: 1 second

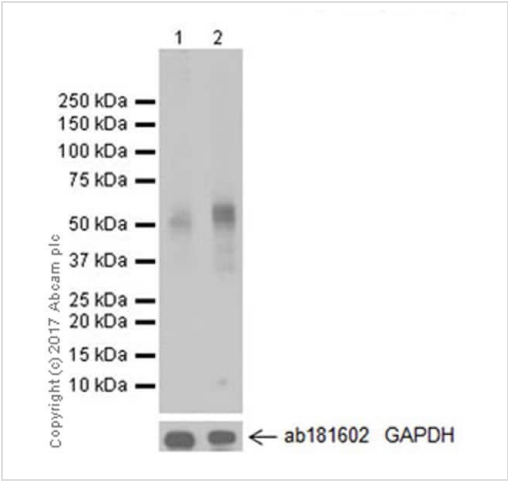
Dilution/blocking buffer: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-PINK1 antibody [EPR20730] (ab216144)

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, **ab141229**) 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, **ab141229**) 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.



Western blot - Anti-PINK1 antibody [EPR20730] (ab216144)

All lanes : Anti-PINK1 antibody [EPR20730] (ab216144) at 1/1000 dilution

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HeLa cells (treated with 10uM carbonyl cyanide 3-chlorophenylhydrazone (CCCP, **ab141229**) for 24 hours) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

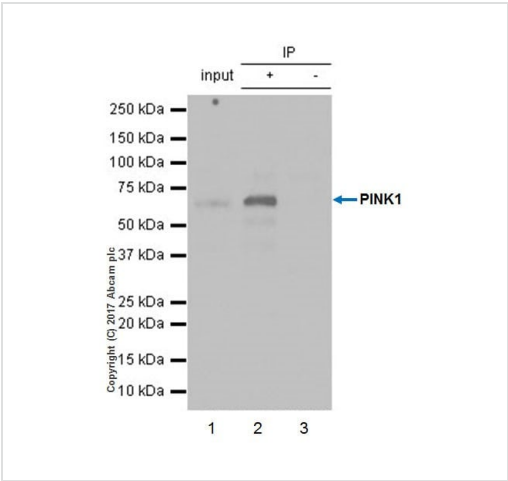
Developed using the ECL technique.

Predicted band size: 63 kDa
Observed band size: 62 kDa

Exposure time: 5 seconds

Blocking and dilution buffer: 5% NFDM/TBST

PINK1 can be induced by CCCP treatment (PMID: 24184327).



Immunoprecipitation - Anti-PINK1 antibody [EPR20730] (ab216144)

PINK1 was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma) (treated with 10uM carbonyl cyanide 3-chlorophenylhydrazone (CCCP. **ab141229**) 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 1/1,000 dilution.

Lane 1: HeLa (CCCP-treated, **ab141229**) lysate 10 µg (Input).
Lane 2: ab216144 IP in HeLa (CCCP-treated, **ab141229**) lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab216144 in HeLa (CCCP-treated, **ab141229**) whole cell lysate.

Blocking and dilution buffer: 5% NFDM/TBST.

Anti-PINK1 antibody [EPR20730] (ab216144)

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