# abcam

#### Product datasheet

## Anti-PINK1 antibody [EPR20730] ab216144



★★★★ 4 Abreviews 14 References

画像数 6

#### 製品の概要

製品名 Anti-PINK1 antibody [EPR20730]

製品の詳細 Rabbit monoclonal [EPR20730] to PINK1

由来種 Rabbit

アプリケーション 適用あり: WB, ICC/IF, IP

種交差性 交差種: Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール 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 3chlorophenylhydrazone (CCCP, ab141229) for 24 hours) whole cell lysate.

特記事項 Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

#### 製品の特性

製品の状態 Liquid

保存方法 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.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS

精製度 Protein A purified

ポリモノ モノクローナル クローン名 EPR20730

アイソタイプ ΙgG

アプリケーション

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## The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab216144の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

| アプリケーション | Abreviews        | 特記事項  |
|----------|------------------|---|
| WB       | <b>★★★★</b> (3)  | 1/1000. Predicted molecular weight: 63 kDa. |
| ICC/IF   | <b>★★★★☆ (1)</b> | 1/500.                                      |
| IP       |                  | 1/30.                                       |

#### ターゲット情報

機能 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.

組織特異性 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.

**関連疾患** 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.

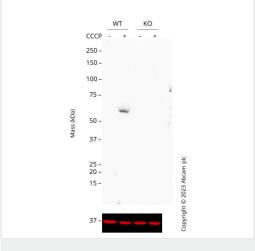
**配列類似性** Belongs to the protein kinase superfamily. Ser/Thr protein kinase family.

Contains 1 protein kinase domain.

翻訳後修飾 Autophosphorylated.

**細胞内局在** Mitochondrion outer membrane. Cytoplasm > cytosol.

### 画像



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, <u>ab141229</u> (0  $\mu$ M, 24h) cell lysate

Lane 2 : Wild-type HEK-293 Treated CCCP,  $\underline{ab141229}$  (10  $\mu\text{M},$  24 h) cell lysate

Lane 3: PINK1 knockout HEK-293 Vehicle Control CCCP, ab141229 (0 µM, 24 h) cell lysate

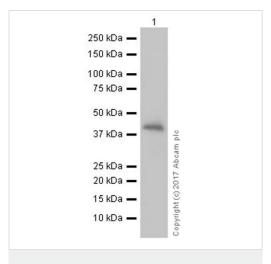
**Lane 4 :** PINK1 knockout HEK-293 Treated CCCP, <u>ab141229</u> (10  $\mu$ M, 24 h) cell lysate

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

ab 216144 DAPI MERGED

ab 216144 DAPI MERGED

Secondary antibody only control on non-treated cells

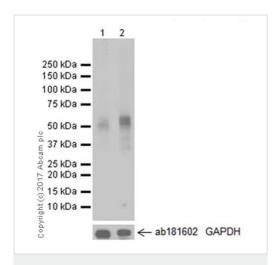
Secondary antibody only control on treated cells

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 lgG 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, <u>ab141229</u>) for 24 hours) whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) 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, <u>ab141229</u>) lysate 10 μg (Input). Lane 2: ab216144 IP in HeLa (CCCP-treated, <u>ab141229</u>) lysate. Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab216144 in HeLa (CCCP-treated, <u>ab141229</u>) whole cell lysate.

Blocking and dilution buffer: 5% NFDM/TBST.



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