# abcam

### Product datasheet

### Anti-PLK1 antibody [EPR19534] ab189139

Recombinant RabMAb

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#### Overview

**Product name** Anti-PLK1 antibody [EPR19534]

**Description** Rabbit monoclonal [EPR19534] to PLK1

**Host species** Rabbit

**Tested applications** Suitable for: Flow Cyt (Intra), ICC/IF, IP, WB

Species reactivity Reacts with: Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa, K562, PC-3 and HepG2 whole cell lysates. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa

and K562 cells. IP: K562 whole cell lysate.

**General notes** 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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

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

**Purity** Protein A purified

Clonality Monoclonal Clone number EPR19534

Isotype lgG

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab189139 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
Flow Cyt (Intra)		1/60. <b>ab172730</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/500.
IP		1/30.
WB		1/1000. Detects a band of approximately 68 kDa (predicted molecular weight: 68 kDa).

#### **Target**

#### **Function**

Serine/threonine-protein kinase that performs several important functions throughout M phase of the cell cycle, including the regulation of centrosome maturation and spindle assembly, the removal of cohesins from chromosome arms, the inactivation of APC/C inhibitors, and the regulation of mitotic exit and cytokinesis. Required for recovery after DNA damage checkpoint and entry into mitosis. Required for kinetochore localization of BUB1B. Phosphorylates SGOL1. Required for spindle pole localization of isoform 3 of SGOL1 and plays a role in regulating its centriole cohesion function. Phosphorylates BORA, and thereby promotes the degradation of BORA. Contributes to the regulation of AURKA function. Regulates TP53 stability through phosphorylation of TOPORS.

#### Tissue specificity

Placenta and colon.

#### Sequence similarities

Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. CDC5/Polo subfamily. Contains 2 POLO box domains.

Contains 1 protein kinase domain.

#### **Developmental stage**

Accumulates to a maximum during the G2 and M phases, declines to a nearly undetectable level following mitosis and throughout G1 phase, and then begins to accumulate again during S phase.

# Post-translational modifications

Catalytic activity is enhanced by phosphorylation of Thr-210. Phosphorylation at Thr-210 is first detected on centrosomes in the G2 phase of the cell cycle, peaks in prometaphase and gradually disappears from centrosomes during anaphase.

Autophosphorylation and phosphorylation of Ser-137 may not be significant for the activation of PLK1 during mitosis, but may enhance catalytic activity during recovery after DNA damage checkpoint.

Ubiquitinated by the anaphase promoting complex/cyclosome (APC/C) in anaphase and following DNA damage, leading to its degradation by the proteasome. Ubiquitination is mediated via its interaction with FZR1/CDH1. Ubiquitination and subsequent degradation prevents entry into

#### Cellular localization

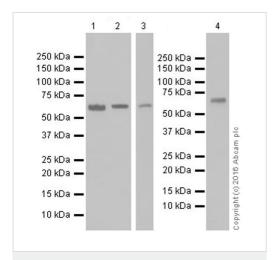
Nucleus. Chromosome > centromere > kinetochore. Cytoplasm > cytoskeleton > centrosome.

During early stages of mitosis, the phosphorylated form is detected on centrosomes and kinetochores. Localizes to the outer kinetochore. Presence of SGOL1 and interaction with the

phosphorylated form of BUB1 is required for the kinetochore localization.

mitosis and is essential to maintain an efficient G2 DNA damage checkpoint.

#### **Images**



Western blot - Anti-PLK1 antibody [EPR19534] (ab189139)

**All lanes :** Anti-PLK1 antibody [EPR19534] (ab189139) at 1/1000 dilution

**Lane 1**: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 2**: K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate

Lane 3 : PC-3 (Human prostate adenocarcinoma cell line) whole cell lysate

**Lane 4 :** HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

#### **Secondary**

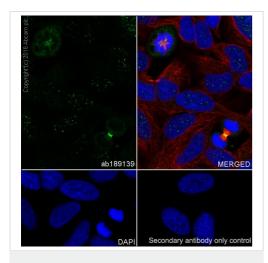
**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 68 kDa Observed band size: 68 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 and 2: 30 seconds; Lane 3 and 4: 3 minutes.

The expression profile observed is consistent with what has been described in the literature (PMID:21545375).



Immunocytochemistry/ Immunofluorescence - Anti-PLK1 antibody [EPR19534] (ab189139)

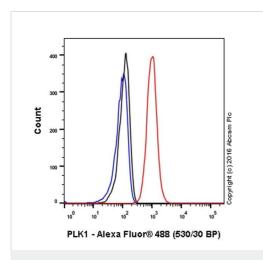
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling PLK1 with ab189139 at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing strong signal staining on midbody and kinetochore of HeLa cell line.

The nuclear counterstain is DAPI (blue).

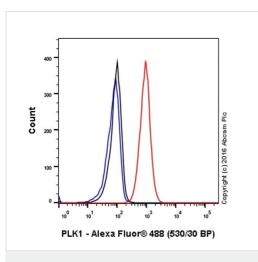
Tubulin is detected with <u>ab195889</u> (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.



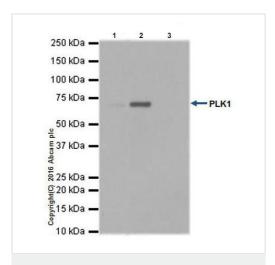
Flow Cytometry (Intracellular) - Anti-PLK1 antibody [EPR19534] (ab189139)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling PLK1with ab189139 at 1/60 dilution (red) compared with a Rabbit IgG,monoclonal [EPR25A]-Isotype control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (Alexa Fluorr<sup>®</sup> 488) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-PLK1 antibody [EPR19534] (ab189139)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed K562 (Human chronic myelogenous leukemia cell line from bone marrow) cells labeling PLK1with ab189139 at 1/60 dilution (red) compared with a Rabbit lgG,monoclonal [EPR25A]-lsotype control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit lgG (Alexa Fluorr® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-PLK1 antibody [EPR19534] (ab189139)

PLK1 was immunoprecipitated from 0.35 mg of K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate with ab189139 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab189139 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

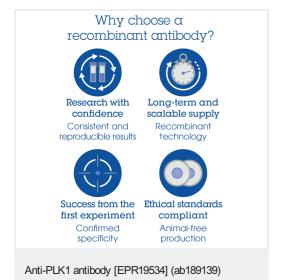
Lane 1: K562 whole cell lysate, 10µg (Input).

Lane 2: ab189139 IP in K562 whole cell lysate.

Lane 3: Rabbit lgG,monoclonal [EPR25A]- Isotype Control (ab172730) instead of ab189139 in K562 whole cell lysate.

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

Exposure time: 30 seconds.



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