abcam

Product datasheet

Anti-PLK1 antibody [36-298] ab17057

3 Abreviews 30 References 6 Images

Overview

Product name	Anti-PLK1 antibody [36-298]
Description	Mouse monoclonal [36-298] to PLK1
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC, WB
Species reactivity	**Reacts with:** Mouse, Human, Recombinant fragment **Predicted to work with:** Rat
Immunogen	Recombinant full length protein corresponding to Human PLK1. His-PLK1 full length purified from Sf9 cells. Database link: **P53350**
Epitope	aa330-370.
Positive control	WB: HEK-293, HeLa S3 or U-2 OS cell lysate ICC: HeLa, HeLa S3, NIH/3T3 or U-2 OS cells Flow Cyt (Intra): HeLa cells.
General notes	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine

Purity	Protein A purified
Clonality	Monoclonal
Clone number	36-298
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab17057 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)		Use 5µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
ICC		Use a concentration of 1 µg/ml.
WB	★★★★☆ **(1)**	Use a concentration of 1 µg/ml. Detects a band of approximately 66 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.

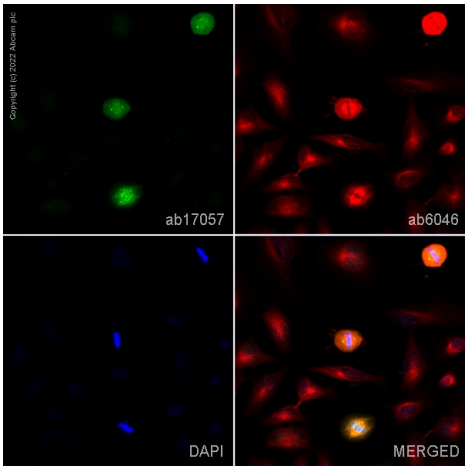
Post-translational modifications Catalytic activity is enhanced by phosphorylation of Thr-210. Phosphorylation at Thr-210 is first detected on centrosomes in the G2 phase of the cell cycle, peaks in prometaphase and gradually disappears from centrosomes during anaphase.
Autophosphorylation and phosphorylation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

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

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.

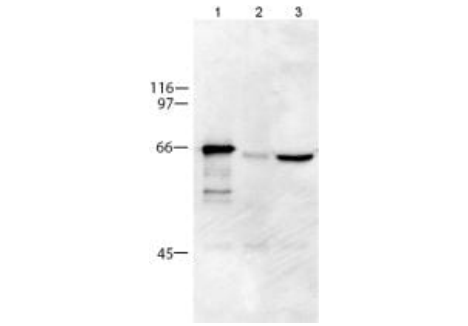
Images



Immunocytochemistry - Anti-PLK1 antibody [36-298] (ab17057)

ab17057 staining PLK1 in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab17057 at 1µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



Western blot - Anti-PLK1 antibody [36-298] (ab17057)

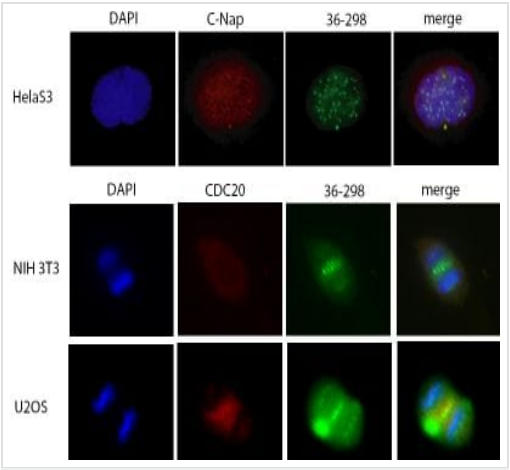
All lanes : Anti-PLK1 antibody [36-298] (ab17057)

Lane 1 : Recombinant PLK1
Lane 2 : U-2 OS (Human bone osteosarcoma epithelial cell line) cell extract
Lane 3 : HeLa S3 cell extract

Performed under reducing conditions.

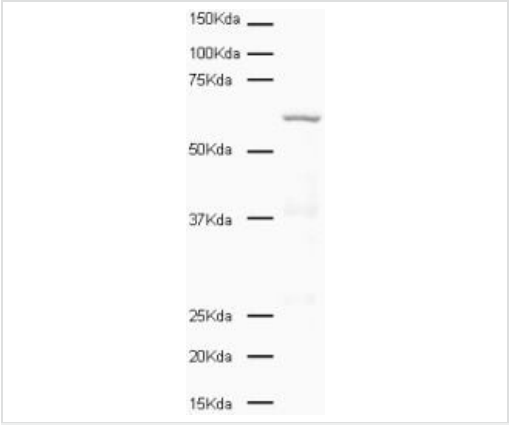
Predicted band size: 68 kDa
Observed band size: 66 kDa

10% SDS-PAGE gel.



Immunocytochemistry - Anti-PLK1 antibody [36-298] (ab17057)

Immunofluoresence using ab17057 and either HeLa S3, NIH/3T3 (Mouse embyro fibroblast cell line) or U-2 OS (Human bone osteosarcoma epithelial cell line) cells.



Western blot - Anti-PLK1 antibody [36-298] (ab17057)

Anti-PLK1 antibody [36-298] (ab17057) at 1 µg/ml + HEK-293 (Human epithelial cell line from embryonic kidney) cell lysate at 20 µg

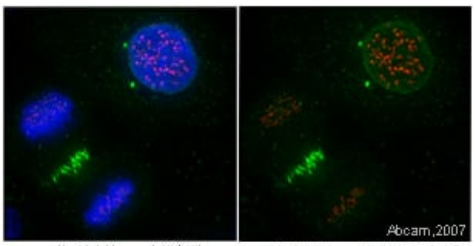
Secondary
Rabbit Anti-Mouse IgG H&L (HRP) (**ab6728**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 68 kDa
Observed band size: 66 kDa

Exposure time: 2 minutes



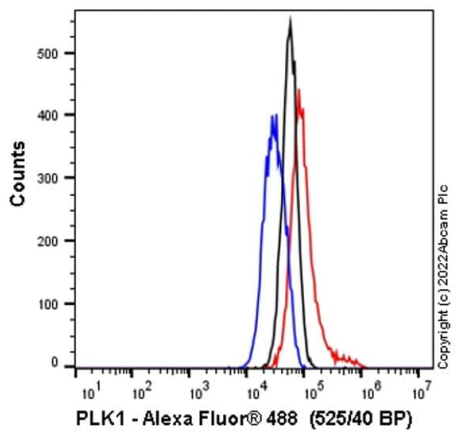
Immunocytochemistry - Anti-PLK1 antibody [36-298] (ab17057)

This image is courtesy of Scott Slattery and Mike Mancini

In panel one HeLa (Human epithelial cell line from cervix adenocarcinoma) cells were stained with ab17057 (green) and DAPI. In the second panel, cells were stained with ab17057 (green) and SH-CREST (red), which stains the centromeres. Fix 30 minutes on ice in 4% formaldehyde in PEM. Quench autofluorescence 2 x 5 minutes with 1 mg/ml Na borohydride or 100 mM ammonium chloride in PEM. Permeabilized for 30 minutes with 0.5% TX-100 in PEM. Block 30 minutes in 5% milk in TBST. Primary antibody incubated overnight at 4°C diluted 1/400 in 5% milk in TBST. Secondary antibody incubated 1 hour at RT diluted in 5% milk in TBST. Post-fix 20 minutes on ice in 4% formaldehyde in PEM. Quench autofluorescence 2 x 5 minutes with ammonium chloride in PEM. Counterstain with DAPI in TBST. Mount with ProLong Gold antifade reagent from Invitrogen.

Notes: Ample washing between each step.

TBST = Tris buffered saline + 0.1% Tween. PEM = 80 mM K-PIPES, pH 6.8, 5 mM EGTA, 2 mM $MgCl_2$.



Flow Cytometry (Intracellular) - Anti-PLK1 antibody [36-298] (ab17057)

Flow cytometry overlay histogram showing HeLa cells stained with ab17057 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab17057) ($1x10^6$ in 100 µl at 5 µg/ml) for 30 min at 22°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/4000 dilution for 30 min at 22°C.

Isotype control antibody (black line) was mouse IgG1 kappa; (**ab170190**) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in HeLa cells fixed with 80% methanol (5 min) / permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors