abcam

Product datasheet

Anti-Chk1 antibody [EP691Y] ab40866

Recombinant RabMAb

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Overview

Product name	Anti-Chk1 antibody [EP691Y]
Description	Rabbit monoclonal [EP691Y] to Chk1
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human Chk1 aa 450-550 (C terminal). The exact sequence is proprietary.
Positive control	HeLa cells lysate and human breast carcinoma.
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**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EP691Y
Isotype	IgG

Applications

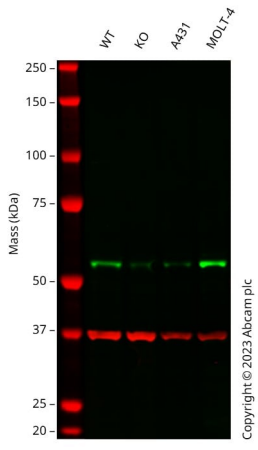
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab40866 in the following tested applications.

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

Application	Abreviews	Notes
Flow Cyt (Intra)		1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/100 - 1/250.
WB	★★★★☆ (5)	1/10000. Detects a band of approximately 54 kDa (predicted molecular weight: 54 kDa).
IHC-P	★★★★★ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Target

Images



Western blot - Anti-Chk1 antibody [EP691Y] (ab40866)

All lanes : Anti-Chk1 antibody [EP691Y] (ab40866) at 1/10000 dilution

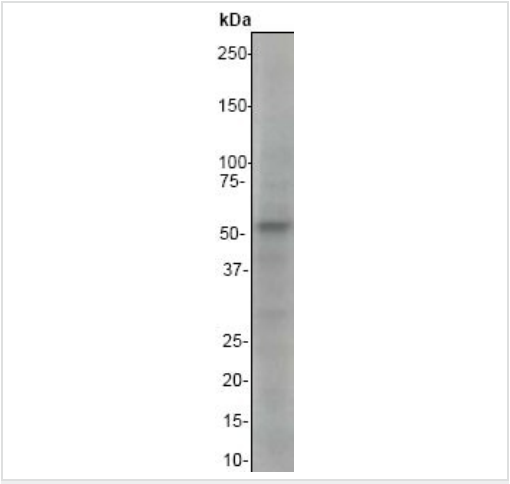
Lane 1 : Wild-type A549 cell lysate
Lane 2 : CHEK1 knockout A549 cell lysate
Lane 3 : A431 cell lysate
Lane 4 : MOLT-4 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 54 kDa
Observed band size: 57 kDa

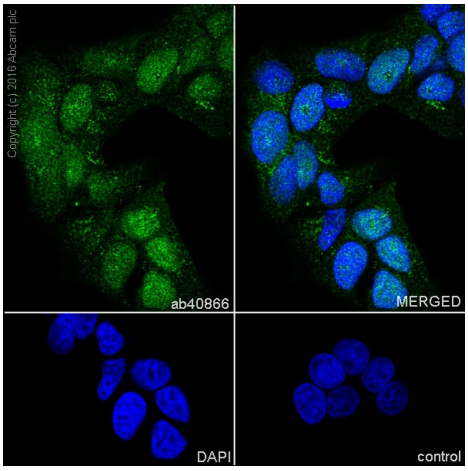
Anti-CHEK1 antibody [EP691Y] (ab40866) staining at 1/10000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab40866 was shown to bind specifically to CHEK1. A band was observed at 57 kDa in wild-type A549 cell lysates with a reduction in signal observed at this size in CHEK1 heterozygous knockout cell line. To generate this image, wild-type and CHEK1 heterozygous knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % 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, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-Chk1 antibody [EP691Y] (ab40866)

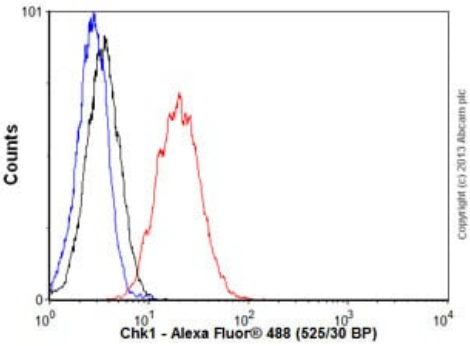
Anti-Chk1 antibody [EP691Y] (ab40866) at 1/10000 dilution + HeLa cell lysate at 10 µg

Predicted band size: 54 kDa
Observed band size: 54 kDa



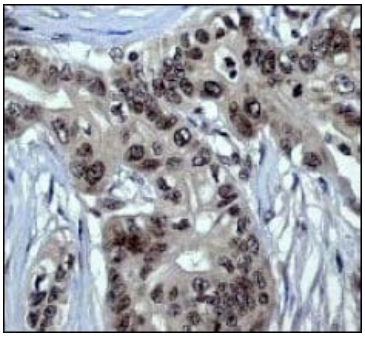
Immunocytochemistry/ Immunofluorescence - Anti-Chk1 antibody [EP691Y] (ab40866)

Immunofluorescence staining of MCF7 cells with purified ab40866 at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was an Alexa Fluor® 488 conjugated goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative control is shown in bottom right hand panel - for the negative control, PBS was used instead of the primary antibody.



Flow Cytometry (Intracellular) - Anti-Chk1 antibody [EP691Y] (ab40866)

Overlay histogram showing HeLa cells stained with ab40866 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab40866, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Chk1 antibody [EP691Y] (ab40866)

Immunohistochemical analysis of Chk1 expression in paraffin-embedded human breast carcinoma using ab40866 at a 1:250 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Anti-Chk1 antibody [EP691Y] (ab40866)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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