abcam

ab40866

Anti-Chk1 antibody [EP691Y]

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP691Y
Purification technique	Affinity purification Protein A
Concentration	1.323 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

WB

Tested

Species	Human
Dilution info	1/10000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/250
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/1000
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target data

See full target information CHEK1

Function Serine/threonine-protein kinase which is required for checkpoint-mediated cell cycle arrest and activation of DNA repair in response to the presence of DNA damage or unreplicated DNA (PubMed:11535615, PubMed:12399544, PubMed:12446774, PubMed:14559997, PubMed:14988723, PubMed:15311285, PubMed:15650047, PubMed:15665856, PubMed:32357935). May also negatively regulate cell cycle progression during unperturbed cell cycles (PubMed:11535615, PubMed:12399544, PubMed:12446774, PubMed:14559997, PubMed:14988723, PubMed:15311285, PubMed:15650047, PubMed:15665856). This regulation is achieved by a number of mechanisms that together help to preserve the integrity of the genome (PubMed:11535615, PubMed:12399544, PubMed:12446774, PubMed:14559997, PubMed:14988723, PubMed:15311285, PubMed:15650047, PubMed:15665856). Recognizes the substrate consensus sequence [R-X-X-S/T] (PubMed:11535615, PubMed:12399544, PubMed:12446774, PubMed:14559997,

PubMed:14988723, PubMed:15311285, PubMed:15650047, PubMed:15665856). Binds to and phosphorylates CDC25A, CDC25B and CDC25C (PubMed:12676583, PubMed:12676925, PubMed:12759351, PubMed:14559997, PubMed:14681206, PubMed:19734889, PubMed:9278511). Phosphorylation of CDC25A at 'Ser-178' and 'Thr-507' and phosphorylation of CDC25C at 'Ser-216' creates binding sites for 14-3-3 proteins which inhibit CDC25A and CDC25C (PubMed:9278511). Phosphorylation of CDC25A at 'Ser-76', 'Ser-124', 'Ser-178', 'Ser-279' and 'Ser-293' promotes proteolysis of CDC25A (PubMed:12676583, PubMed:12676925, PubMed:12759351, PubMed:14681206, PubMed:19734889, PubMed:9278511). Phosphorylation of CDC25A at 'Ser-76' primes the protein for subsequent phosphorylation at 'Ser-79', 'Ser-82' and 'Ser-88' by NEK11, which is required for polyubiquitination and degradation of CDCD25A (PubMed:19734889, PubMed:20090422, PubMed:9278511). Inhibition of CDC25 leads to increased inhibitory tyrosine phosphorylation of CDK-cyclin complexes and blocks cell cycle progression (PubMed:9278511). Also phosphorylates NEK6 (PubMed:18728393). Binds to and phosphorylates RAD51 at 'Thr-309', which promotes the release of RAD51 from BRCA2 and enhances the association of RAD51 with chromatin, thereby promoting DNA repair by homologous recombination (PubMed:15665856). Phosphorylates multiple sites within the C-terminus of TP53, which promotes activation of TP53 by acetylation and promotes cell cycle arrest and suppression of cellular proliferation (PubMed:10673501, PubMed:15659650, PubMed:16511572). Also promotes repair of DNA cross-links through phosphorylation of FANCE (PubMed:17296736). Binds to and phosphorylates TLK1 at 'Ser-743', which prevents the TLK1-dependent phosphorylation of the chromatin assembly factor ASF1A (PubMed:12660173, PubMed:12955071). This may enhance chromatin assembly both in the presence or absence of DNA damage (PubMed:12660173, PubMed:12955071). May also play a role in replication fork maintenance through regulation of PCNA (PubMed:18451105). May regulate the transcription of genes that regulate cell-cycle progression through the phosphorylation of histones (By similarity). Phosphorylates histone H3.1 (to form H3T11ph), which leads to epigenetic inhibition of a subset of genes (By similarity). May also phosphorylate RB1 to promote its interaction with the E2F family of transcription factors and subsequent cell cycle arrest (PubMed:17380128). Phosphorylates SPRTN, promoting SPRTN recruitment to chromatin (PubMed:31316063). Reduces replication stress and activates the G2/M checkpoint, by phosphorylating and inactivating PABIR1/FAM122A and promoting the serine/threonine-protein phosphatase 2A-mediated dephosphorylation and stabilization of WEE1 levels and activity (PubMed:33108758). Isoform 2. Endogenous repressor of isoform 1, interacts with, and antagonizes CHK1 to promote the S to G2/M phase transition.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C

Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

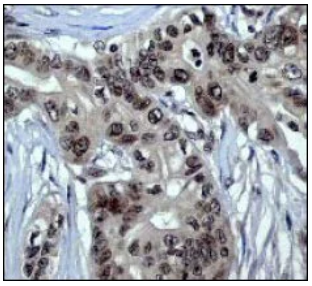
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

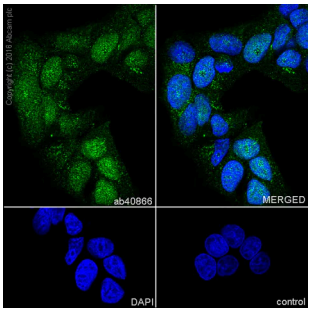
6 product images



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

Chk1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Chk1 antibody

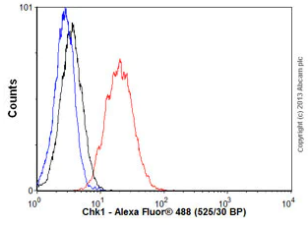
Immunohistochemical analysis of Chk1 expression in paraffin-embedded human breast carcinoma using ab40866 at a 1:250 dilution.
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

Chk1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Chk1 antibody

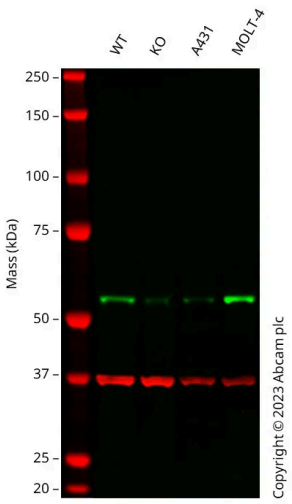
Immunofluorescence staining of MCF7 cells with purified ab40866 at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was an Alexa Fluor® 488 conjugated goat anti-rabbit (ab150077), used at a dilution of 1/1000. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton 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)

Chk1 Flow Cytometry (Intracellular) staining using rabbit Anti-Chk1 antibody

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.



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

Chk1 Western blot staining using rabbit Anti-Chk1 antibody

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

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
CHEK1 knockout A549 cell lysate at 20 µg

Lane 3:
A431 cell lysate at 20 µg

Lane 4:
MOLT-4 cell lysate at 20 µg

Secondary

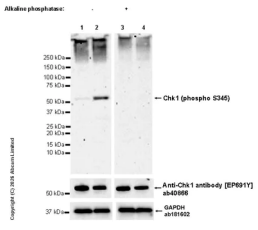
Lanes 1 - 4:
Goat anti-Rabbit IgG H&L 800CW at 1/20000 dilution

Lanes 1 - 4:
Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 54 kDa

Observed band size: 57 kDa



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

Chk1 Western blot staining using rabbit Anti-Chk1 antibody

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

UV-C treatment induces phosphorylation of Chk1 at Ser345 (PMID: 20609246).

The identity of the bands higher than 150 kDa are unknown.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-Chk1 antibody [EP691Y] (ab40866) staining at 56KDa dilution.

All lanes:
Western blot - Anti-Chk1 (phospho S345) antibody [EPR30270-566] (ab325675) at 1/1000 dilution

Lane 1:
Untreated Synchronized G1/S HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate (untreated membrane) at 20 µg

Lane 2:
Synchronized G1/S HeLa treated with 100 J/m2 UV-C, then recovery for 1 hour whole cell lysate (untreated membrane) at 20 µg

Lane 3:
Untreated Synchronized G1/S HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate (phosphatase treated membrane) at 20 µg

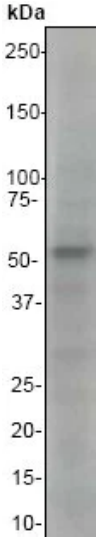
Lane 4:
Synchronized G1/S HeLa treated with 100 J/m2 UV-C, then recovery for 1 hour whole cell lysate (phosphatase treated membrane) at 20 µg

Secondary

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

Observed band size: 56 kDa, 36 kDa

Exposure time: 180s



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

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

All lanes:
HeLa cell lysate at 10 µg

Predicted band size: 54 kDa

Observed band size: 54 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.