abcam

ab227998

Anti-Chk2 antibody [EPR4325] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR4325
Purification technique	Affinity purification Protein A
Concentration	1.04 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-
Notes	antigen retrieval is recommended.

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.

Target data

See full target information CHEK2

Function Serine/threonine-protein kinase which is required for checkpoint-mediated cell cycle arrest, activation of DNA repair and apoptosis in response to the presence of

DNA double-strand breaks. May also negatively regulate cell cycle progression during unperturbed cell cycles. Following activation, phosphorylates numerous effectors preferentially at the consensus sequence [L-X-R-X-X-S/T] (PubMed:37943659). Regulates cell cycle checkpoint arrest through phosphorylation of CDC25A, CDC25B and CDC25C, inhibiting their activity. Inhibition of CDC25 phosphatase activity leads to increased inhibitory tyrosine phosphorylation of CDK-cyclin complexes and blocks cell cycle progression. May also phosphorylate NEK6 which is involved in G2/M cell cycle arrest. Regulates DNA repair through phosphorylation of BRCA2, enhancing the association of RAD51 with chromatin which promotes DNA repair by homologous recombination. Also stimulates the transcription of genes involved in DNA repair (including BRCA2) through the phosphorylation and activation of the transcription factor FOXM1. Regulates apoptosis through the phosphorylation of p53/TP53, MDM4 and PML. Phosphorylation of p53/TP53 at 'Ser-20' by CHEK2 may alleviate inhibition by MDM2, leading to accumulation of active p53/TP53. Phosphorylation of MDM4 may also reduce degradation of p53/TP53. Also controls the transcription of pro-apoptotic genes through phosphorylation of the transcription factor E2F1. Tumor suppressor, it may also have a DNA damage-independent function in mitotic spindle assembly by phosphorylating BRCA1. Its absence may be a cause of the chromosomal instability observed in some cancer cells. Promotes the CCAR2-SIRT1 association and is required for CCAR2-mediated SIRT1 inhibition (PubMed:25361978). Under oxidative stress, promotes ATG7 ubiquitination by phosphorylating the E3 ubiquitin ligase TRIM32 at 'Ser-55' leading to positive regulation of the autophagosme assembly (PubMed:37943659). (Microbial infection) Phosphorylates herpes simplex virus 1/HHV-1 protein ICP0 and thus activates its SUMO-targeted ubiquitin ligase activity.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab227998 is the carrier-free version of ab109413.

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.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes,

oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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

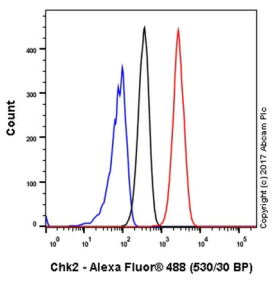
We do not recommend this combination. It is not covered by our product promise.

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.

9 product images

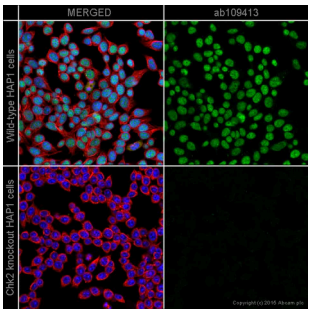


Flow Cytometry (Intracellular) - Anti-Chk2 antibody [EPR4325] - BSA and Azide free (ab227998)

Chk2 Flow Cytometry (Intracellular) staining using rabbit Anti-Chk2 antibody

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling Chk2 with purified ab109413 at 1/230 dilution (10 µg/ml) (red). Cells were fixed with 80% methanol and permeabilised with 0.1% Tween-20. A Goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) (ab172730) was used

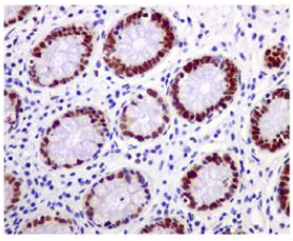
as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109413).



Immunocytochemistry/ Immunofluorescence - Anti-Chk2 antibody [EPR4325] - BSA and Azide free (ab227998)

Chk2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Chk2 antibody

ab109413 staining Chk2 in wild-type HAP1 cells (top panel) and Chk2 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5 minutes), permeabilized with 0.1% 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 1 hour. The cells were then incubated with ab109413 at 1/250 dilution and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1 hour with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109413).

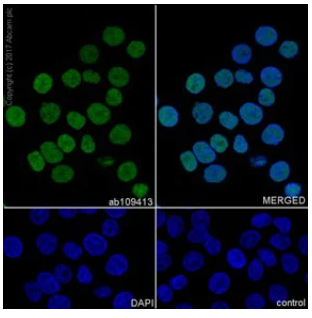


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Chk2 antibody [EPR4325] - BSA and Azide free (ab227998)

Chk2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Chk2 antibody

This IHC data was generated using the same anti-Chk2 antibody clone, EPR4325, in a different buffer formulation (cat# ab109413).
Immunohistochemical analysis of paraffin-embedded human colon tissue using ab109413 at a 1/100 dilution.

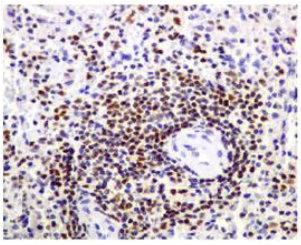
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Chk2 antibody [EPR4325] - BSA and Azide free (ab227998)

Chk2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Chk2 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab109413).
Immunocytochemistry analysis of HT-29 (human colorectal adenocarcinoma epithelial cell) labeling Chk2 with purified ab109413 at 1/500 dilution. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% tritonX-100. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/1000 (2 µg/ml) was used as the secondary antibody. was used as counterstain. Nuclei were stained blue with DAPI.
Negative control: PBS instead of the primary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Chk2 antibody [EPR4325] - BSA and Azide free (ab227998)

Chk2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Chk2 antibody

Immunohistochemical analysis of paraffin-embedded human spleen tissue using ab109413 at a 1/100 dilution.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109413).

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

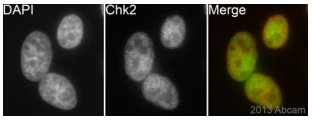
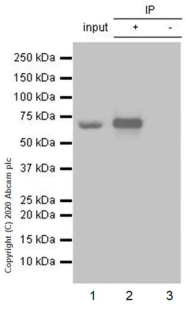


Image courtesy of a customer review submitted by Dr. Kirk McManus, Univ. of Manitoba/Cancer Care MICB, Canada

Immunocytochemistry/ Immunofluorescence - Anti-Chk2 antibody [EPR4325] - BSA and Azide free (ab227998)

Chk2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Chk2 antibody

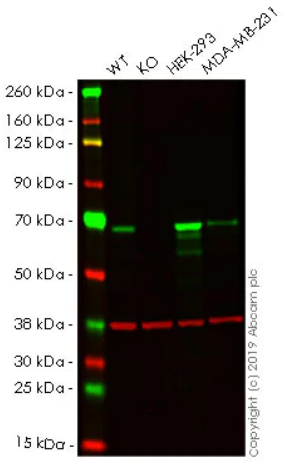
ab109413 (1/500) staining Chk2 in HeLa (human epithelial cell line from cervix adenocarcinoma) cells (green). Cells were fixed in paraformaldehyde, permeabilized with 0.5% Triton X-100/PBS and counterstained with DAPI in order to highlight the nucleus (red). For further experimental details please see Abreview.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109413).



Immunoprecipitation - Anti-Chk2 antibody [EPR4325] - BSA and Azide free (ab227998)

This data was developed using ab109413, the same antibody clone in a different buffer formulation.
Purified ab109413 at 1/50 dilution (2µg) immunoprecipitating Chk2 in HeLa whole cell lysate.
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab109413 + HeLa whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab109413 in HeLa whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (ab131366) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 62 kDa
All lanes:
Immunoprecipitation - Anti-Chk2 antibody [EPR4325] (ab109413)

Predicted band size: 61 kDa



Western blot - Anti-Chk2 antibody [EPR4325] - BSA and Azide free (ab227998)

Chk2 Western blot staining using rabbit Anti-Chk2 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab109413).
Lanes 1-4: Merged signal (red and green). Green - ab109413 observed at 68 kDa. Red - loading control ab8245 observed at 37 kDa.

ab109413 Anti-Chk2 antibody [EPR4325] was shown to specifically react with Chk2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab264815 (knockout cell lysate ab257104) was used. Wild-type and Chk2 knockout samples were subjected to SDS-PAGE. ab109413 and Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-Chk2 antibody [EPR4325] (ab109413) at 1/1000 dilution

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

Lane 2:
CHEK2 knockout HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human CHEK2 (Chk2) knockout HeLa cell line (ab264815)

Lane 3:
HEK-293 cell lysate at 20 µg

Lane 4:
MDA-MB-231 cell lysate at 20 µg

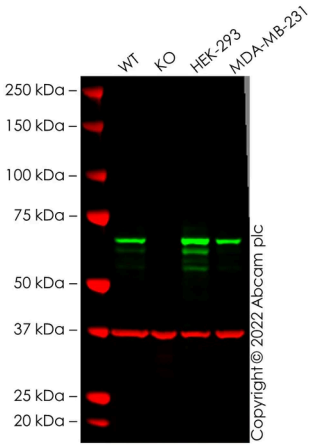
Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 61 kDa

Observed band size: 68 kDa



Western blot - Anti-Chk2 antibody [EPR4325] - BSA and Azide free (ab227998)

This data was developed using the same antibody clone in a different buffer formulation (ab109413) False colour image of Western blot: Anti-Chk2 antibody [EPR4325] staining at 1/50000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab109413 was shown to bind specifically to Chk2. A band was observed at 67 kDa in wild-type A549 cell lysates with no signal observed at this size in CHEK2 knockout cell line ab276098 (knockout cell lysate ab276098). To generate this image, wild-type and CHEK2 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.
Lanes 1 - 4:
Western blot - Anti-Chk2 antibody [EPR4325] (ab109413) at 1/5000 dilution

Lanes 1 - 4:
Western blot - Anti-Chk2 antibody [EPR4325] - BSA and Azide free (ab227998) at 1/5000 dilution

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

Lane 2:
Western blot - Human CHEK2 (Chk2) knockout A549 cell line (ab276098)

Lane 2:
CHEK2 knockout A549 cell lysate at 20 µg

Lane 3:
HEK-293 cell lysate at 20 µg

Lane 4:
MDA-MB-231 cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 67 kDa

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