abcam

Product datasheet

# Anti-Chk2 antibody [EPR4325] - BSA and Azide free ab227998

KO VALIDATED Recombinant RabMAb

4 References 11 Images

## Overview

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|---|---|
| **Product name** | Anti-Chk2 antibody [EPR4325] - BSA and Azide free |
| **Description** | Rabbit monoclonal [EPR4325] to Chk2 - BSA and Azide free |
| **Host species** | Rabbit |
| **Tested applications** | **Suitable for:** IP, IHC-P, WB, Flow Cyt (Intra), ICC/IF |
| **Species reactivity** | **Reacts with:** Human |
| **Immunogen** | Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. |
| **Positive control** | WB: HeLa (untreated and treated with gamma irradiation), HEK-293, MDA-MB-231, HT-29, and 293T cell lysates. IHC-P: Human colon and spleen tissues. ICC/IF: Wild-type HAP1 cells. IP: HeLa whole cell lysate. |
| **General notes** | ab227998 is the carrier-free version of **ab109413**.<br><br>Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.<br><br>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.<br><br>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.<br><br>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.<br><br>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

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| **Form** | Liquid |

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| **Storage instructions** | Shipped at 4°C. Store at +4°C. Do Not Freeze. |
| **Storage buffer** | pH: 7.20<br>Constituent: PBS |
| **Carrier free** | Yes |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | EPR4325 |
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab227998 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 |
|---|---|---|
| **IP** | | Use at an assay dependent concentration. |
| **IHC-P** | | Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.<br>antigen retrieval is recommended. |
| **WB** | | Use at an assay dependent concentration. Detects a band of approximately 62 kDa (predicted molecular weight: 61 kDa). |
| **Flow Cyt (Intra)** | | Use at an assay dependent concentration.<br>**ab199376** - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody. |
| **ICC/IF** | | Use at an assay dependent concentration. |

## Target

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| **Function** | Regulates cell cycle checkpoints and apoptosis in response to DNA damage, particularly to DNA double-strand breaks. Inhibits CDC25C phosphatase by phosphorylation on 'Ser-216', preventing the entry into mitosis. May also play a role in meiosis. Regulates the TP53 tumor suppressor through phosphorylation at 'Thr-18' and 'Ser-20'. |
| **Tissue specificity** | High expression is found in testis, spleen, colon and peripheral blood leukocytes. Low expression is found in other tissues. |
| **Involvement in disease** | Defects in CHEK2 are associated with Li-Fraumeni syndrome 2 (LFS2) [MIM:609265]; a highly penetrant familial cancer phenotype usually associated with inherited mutations in p53/TP53. Defects in CHEK2 may be a cause of susceptibility to prostate cancer (PC) [MIM:176807]. It is a malignancy originating in tissues of the prostate. Most prostate cancers are adenocarcinomas that develop in the acini of the prostatic ducts. Other rare histopathologic types of prostate cancer that occur in approximately 5% of patients include small cell carcinoma, mucinous carcinoma, prostatic ductal carcinoma, transitional cell carcinoma, squamous cell carcinoma, basal cell |

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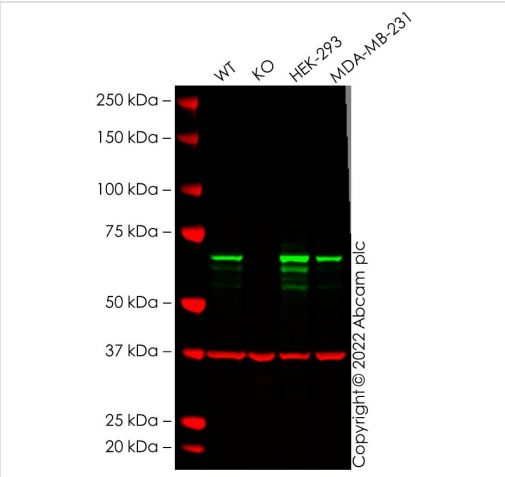
| | carcinoma, adenoid cystic carcinoma (basaloid), signet-ring cell carcinoma and neuroendocrine carcinoma.<br>Defects in CHEK2 are found in some patients with osteogenic sarcoma (OSRC) [MIM:259500]. |
| **Sequence similarities** | Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. CHK2 subfamily.<br>Contains 1 FHA domain.<br>Contains 1 protein kinase domain. |
| **Post-translational modifications** | Phosphorylated by PLK4. |
| **Cellular localization** | Nucleus; Nucleus. Isoform 10 is present throughout the cell and Nucleus > PML body. Nucleus > nucleoplasm. Recruited into PML bodies together with TP53. |

## Images




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

**All lanes :** Anti-Chk2 antibody [EPR4325] (**ab109413**) at 1/50000 dilution

**Lane 1 :** Wild-type A549 cell lysate
**Lane 2 :** CHEK2 knockout A549 cell lysate
**Lane 3 :** HEK-293 cell lysate
**Lane 4 :** MDA-MB-231 cell lysate

Lysates/proteins at 20 µg per lane.

**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.

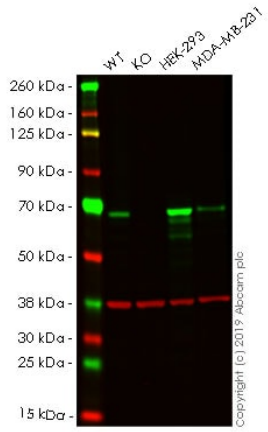
**Predicted band size:** 61 kDa
**Observed band size:** 67 kDa

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.




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

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

**Lane 1 :** Wild-type HeLa cell lysate
**Lane 2 :** CHEK2 knockout HeLa cell lysate
**Lane 3 :** HEK-293 cell lysate
**Lane 4 :** MDA-MB-231 cell lysate

Lysates/proteins at 20 µg per lane.

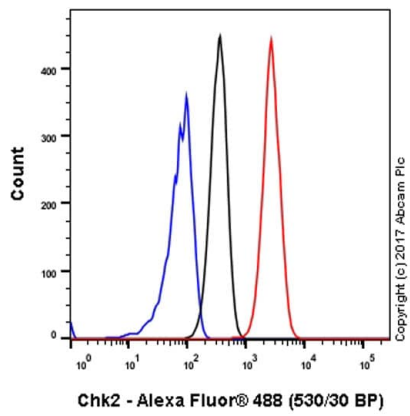
Performed under reducing conditions.

**Predicted band size:** 61 kDa
**Observed band size:** 68 kDa

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.

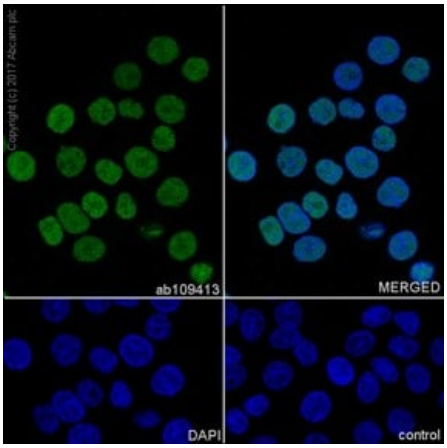
**ab109413** Anti-Chk2 antibody [EPR4325] was shown to specifically react with Chk2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab264815** (knockout cell lysate **ab257104**) was used. Wild-type and Chk2 knockout samples were subjected to SDS-PAGE. **ab109413** and 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.




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

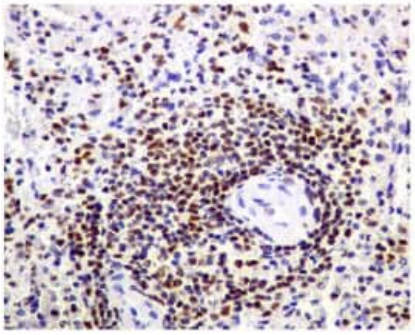
Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling Chk2 with purified **ab109413** at 1/230 dilution (10 µg/ml) (red). Cells were fixed with 80% methanol and permeabilised with 0.1% Tween-20. A Goat anti rabbit IgG (Alexa Fluor® 488) (**ab150077**) (1/2000 dilution)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

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.

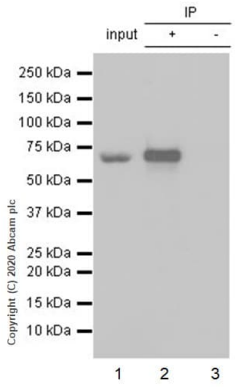


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Chk2 antibody [EPR4325] - BSA and Azide free (ab227998)

Immunohistochemical analysis of paraffin-embedded human spleen tissue using **ab109413** at a 1/100 dilution.

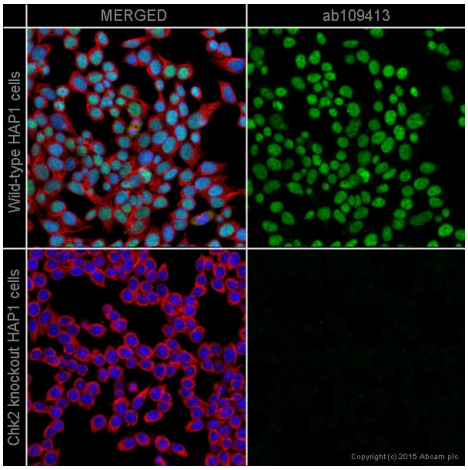
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109413**).

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




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

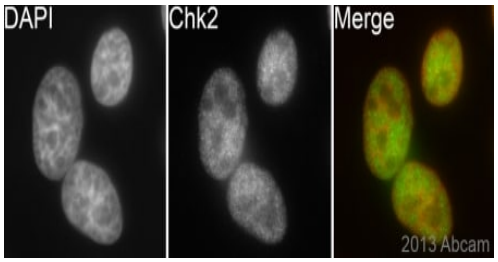
This data was developed using **ab109413**, the same antibody clone in a different buffer formulation.
Purified **ab109413** at 1/50 dilution (2µg) immunoprecipitating Chk2 in HeLa whole cell lysate.
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): **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




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

**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**).

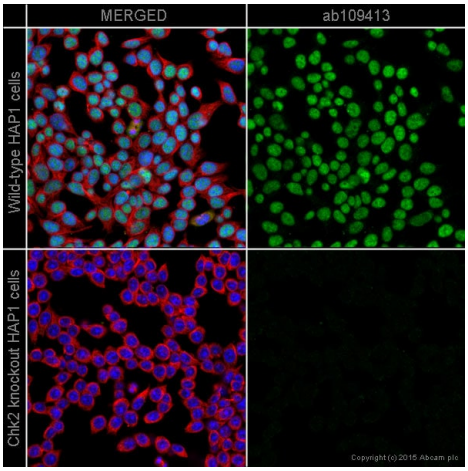



Immunocytochemistry/ Immunofluorescence - Anti-Chk2 antibody [EPR4325] - BSA and Azide free (ab227998)

This image is courtesy of an Abreview submitted by Kirk McManus.

**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.

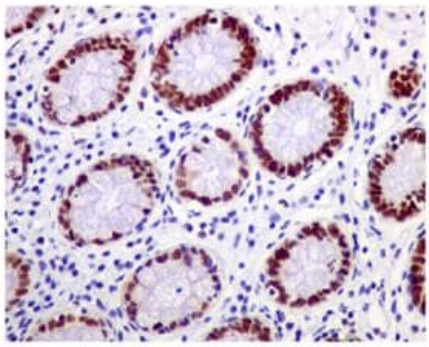
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109413**).




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

This ICC data was generated using the same anti-Chk2 antibody clone, EPR4325, in a different buffer formulation (cat# **ab10413**).

**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.

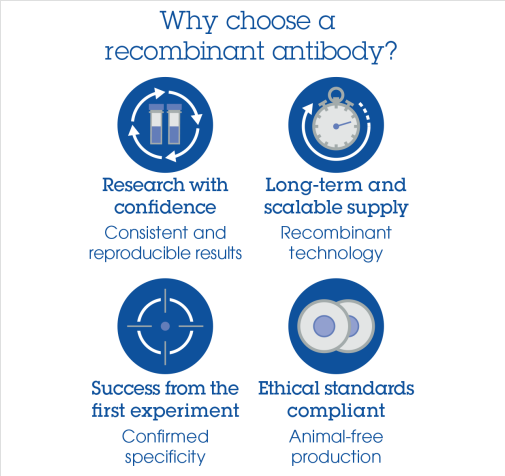


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Chk2 antibody [EPR4325] - BSA and Azide free (ab227998)

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.




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

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