abcam

Product datasheet

Anti-Chk2 antibody [EPR4325] ab109413

KO 評価済 リコンビナント RabMAb

★★★★★ 9 Abreviews 21 References 画像数 13

製品の概要

製品名	Anti-Chk2 antibody [EPR4325]
製品の詳細	Rabbit monoclonal [EPR4325] to Chk2
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), ICC/IF, WB, IP, IHC-P
種交差性	**交差種:** Human
免疫原	Recombinant fragment within Human Chk2 aa 1-200. The exact sequence is proprietary. Database link: **O96017**
ポジティブ・コントロール	WB: HeLa (untreated and treated with gamma irradiation), HAP1, CHEK2, HEK293, MDA-MB-231, HT-29, and 293T cell lysates. IHC-P: Human colon and spleen tissues. ICC/IF: Wild-type HAP1 cells. Flow Cyt (intra): HeLa IP: HeLa whole cell lysate.
特記事項	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.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
バッファー	pH: 7.20 Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant

精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR4325
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab109413の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

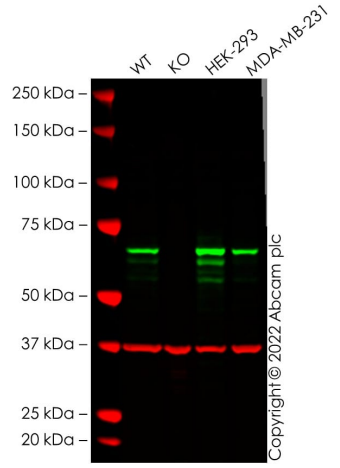
アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (4)	1/100 - 1/250.
WB	★★★★★ (5)	1/50000 - 1/200000. Detects a band of approximately 62 kDa (predicted molecular weight: 61 kDa).
IP		1/10 - 1/100.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. antigen retrieval is recommended.

ターゲット情報

機能	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'.
組織特異性	High expression is found in testis, spleen, colon and peripheral blood leukocytes. Low expression is found in other tissues.
関連疾患	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 carcinoma, adenoid cystic carcinoma (basaloid), signet-ring cell carcinoma and neuroendocrine carcinoma. Defects in CHEK2 are found in some patients with osteogenic sarcoma (OSRC) [MIM:259500].
配列類似性	Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. CHK2 subfamily. Contains 1 FHA domain. Contains 1 protein kinase domain.

翻訳後修飾	Phosphorylated by PLK4.
細胞内局在	Nucleus; Nucleus. Isoform 10 is present throughout the cell and Nucleus > PML body. Nucleus > nucleoplasm. Recruited into PML bodies together with TP53.

画像



Western blot - Anti-Chk2 antibody [EPR4325] (ab109413)

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.

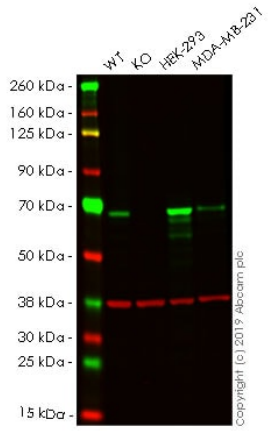
Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

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

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] (ab109413)

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

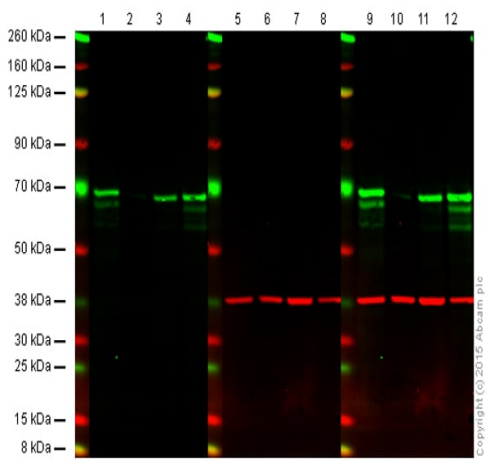
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

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.



Western blot - Anti-Chk2 antibody [EPR4325] (ab109413)

Lanes 1-4 : Anti-Chk2 antibody [EPR4325] (ab109413) at 1/50000 dilution
Lanes 5-8 : Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) at 1/2000 dilution

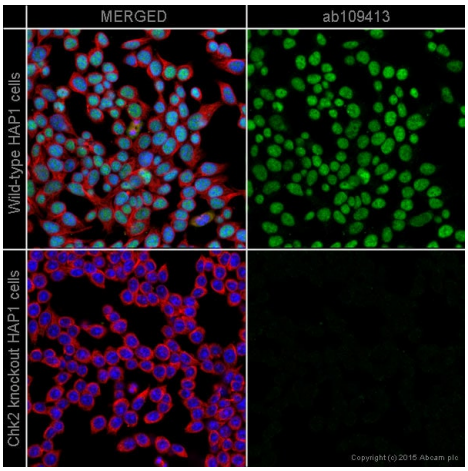
Lanes 1 & 5 : Wild-type HAP1 cell lysate
Lanes 2 & 6 : Chk2 knockout HAP1 cell lysate
Lanes 3 & 7 : HeLa cell lysate
Lanes 4 & 8 : HEK293 cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 61 kDa

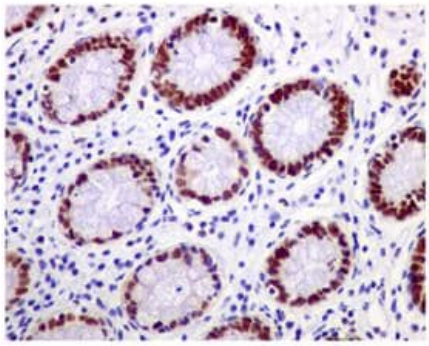
Lanes 1, 2, 3 and 4: Green signal from target - ab109413 observed at 62 kDa
Lanes 5, 6, 7 and 8: Red signal from loading control - **ab8245** observed at 37 kDa
Lanes 9, 10, 11 and 12: Merged (red and green) signal

ab109413 was shown to specifically react with Chk2 when Chk2 knockout samples were used. Wild-type and Chk2 knockout samples were subjected to SDS-PAGE. ab109413 and **ab8245** (loading control to GAPDH) were diluted 1/50 000 and 1/2000 respectively and incubated overnight at 4°C. 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 1/10 000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Chk2 antibody [EPR4325] (ab109413)

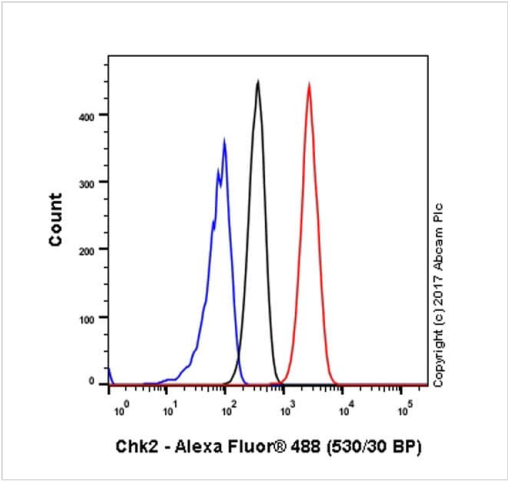
ab109413 staining Chk2 in wild-type HAP1 cells (top panel) and Chk2 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5 minutes), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1 hour. 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] (ab109413)

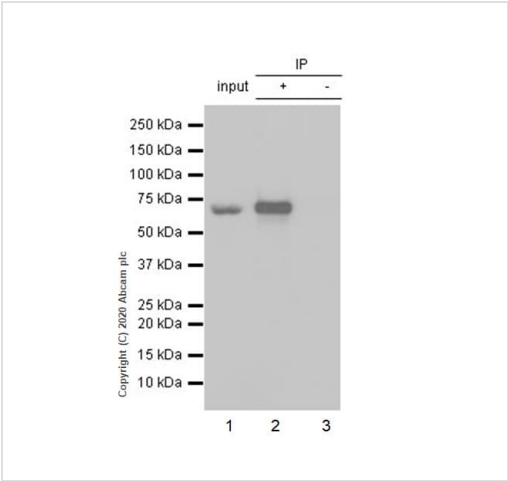
Immunohistochemical analysis of paraffin-embedded human colon tissue using ab109413 at a 1/100 dilution.

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



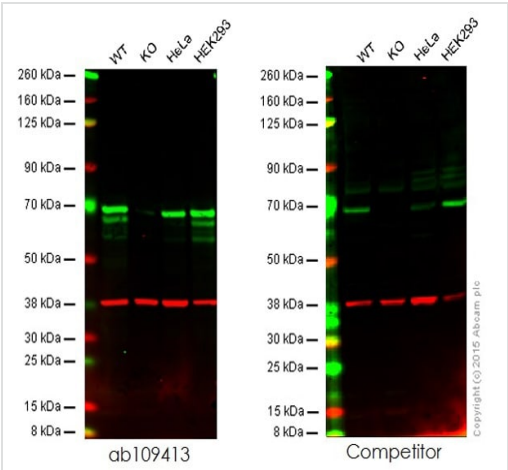
Flow Cytometry (Intracellular) - Anti-Chk2 antibody [EPR4325] (ab109413)

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



Immunoprecipitation - Anti-Chk2 antibody [EPR4325] (ab109413)

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



Western blot - Anti-Chk2 antibody [EPR4325] (ab109413)

All lanes : Anti-Chk2 antibody [EPR4325] (ab109413)

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : Chk2 knockout HAP1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : HEK293 cell lysate

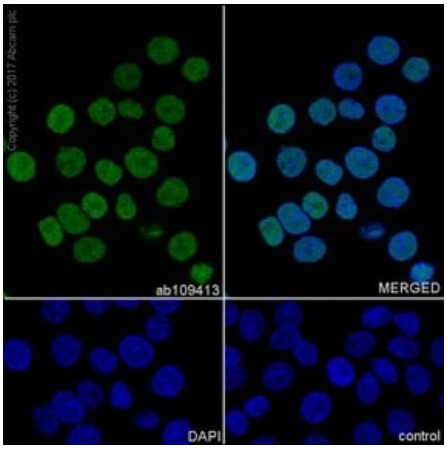
Lysates/proteins at 20 µg per lane.

Predicted band size: 61 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab109413 observed at 64 kDa. Red - loading control, **ab8245**, observed at

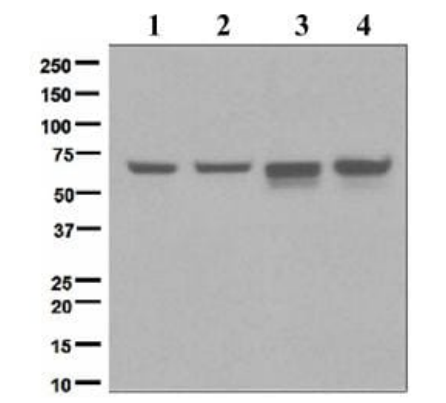
37 kDa.

This western blot image is a comparison between ab109413 and a competitor's rabbit polyclonal antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Chk2 antibody [EPR4325] (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.



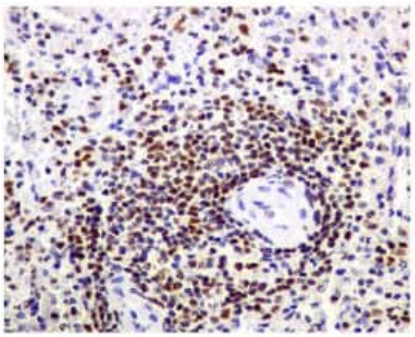
Western blot - Anti-Chk2 antibody [EPR4325] (ab109413)

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

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) cells treated with gamma irradiation
Lane 2 : HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 3 : HT-29 (human colorectal adenocarcinoma cell line) cell lysate
Lane 4 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cell lysate

Lysates/proteins at 10 µg per lane.

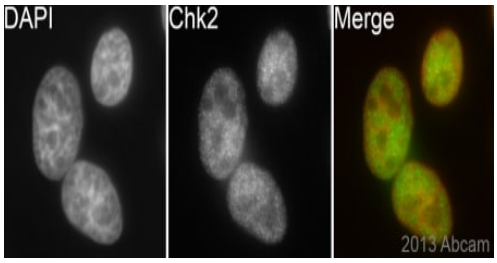
Predicted band size: 61 kDa
Observed band size: 62 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Chk2 antibody [EPR4325] (ab109413)

Immunohistochemical analysis of paraffin-embedded human spleen 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] (ab109413)

Image courtesy of an Abreview submitted by Dr. Kirk McManus, Univ. of Manitoba/Cancer Care MICB, Canada

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.

Anti-Chk2 antibody [EPR4325] (ab109413)

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

Our Abpromise to you: Quality guaranteed and expert technical support

- 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.co.jp/abpromise** or contact our technical team.

Terms and conditions

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