abcam

Product datasheet

Anti-Chk2 antibody [EPR19482] ab207446

KO VALIDATED Recombinant RabMAb

★★★★★ 1 Abreviews 2 References 13 Images

Overview

Product name	Anti-Chk2 antibody [EPR19482]
Description	Rabbit monoclonal [EPR19482] to Chk2
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HeLa, HEK-293, HEK-293T, MDA-MB-231, HCT 116 and SH-SY5Y whole cell lysates; human colon, thymus and kidney lysates. IHC-P: Human testis and breast cancer tissues. ICC/IF: HeLa and HCT 116 cells. Flow Cyt (intra): HCT 116 cells. IP: HeLa and HEK-293T whole cell lysates.
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**.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19482

Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab207446 in the following tested applications.

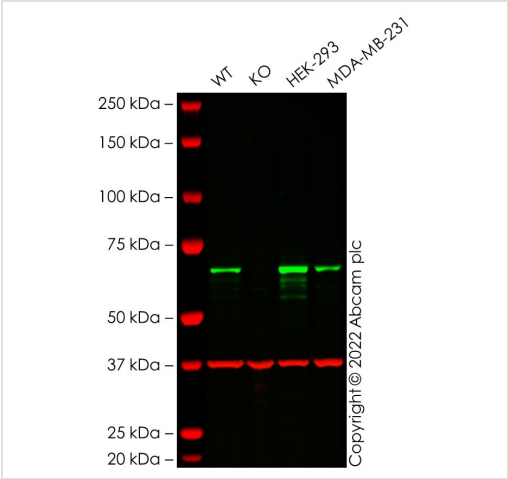
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/70.
WB	★★★★★ (1)	1/1000. Detects a band of approximately 61 kDa (predicted molecular weight: 61 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/500.
IP		1/40.

Target

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 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].
Sequence similarities	Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. CHK2 subfamily. Contains 1 FHA domain. 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 [EPR19482] (ab207446)

All lanes : Anti-Chk2 antibody [EPR19482] (ab207446) at 1/1000 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.

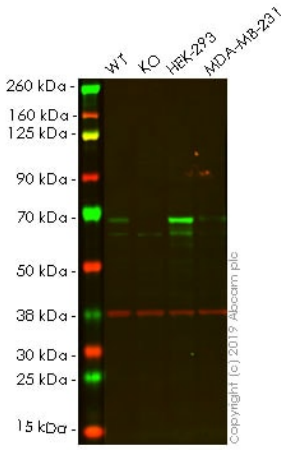
Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

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

False colour image of Western blot: Anti-Chk2 antibody [EPR19482] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab207446 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 [EPR19482] (ab207446)

All lanes : Anti-Chk2 antibody [EPR19482] (ab207446) 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

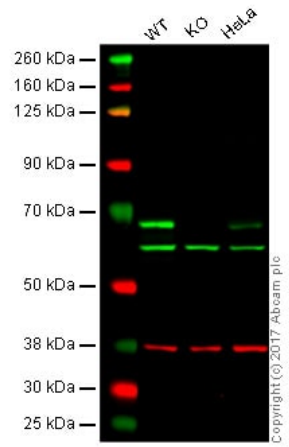
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Lanes 1-4: Merged signal (red and green). Green - ab207446 observed at 68 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab207446 Anti-Chk2 antibody [EPR19482] 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. ab207446 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 [EPR19482] (ab207446)

All lanes : Anti-Chk2 antibody [EPR19482] (ab207446) at 1/1000 dilution

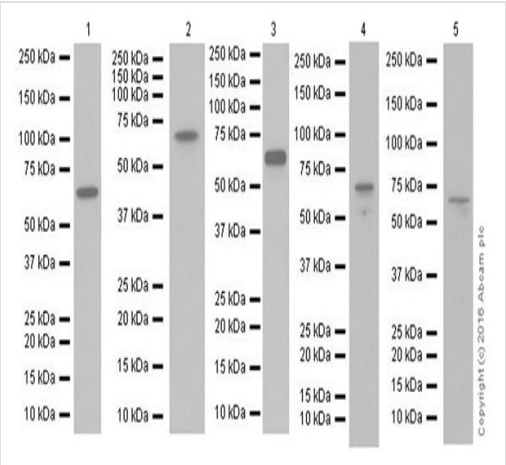
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : CHEK2 knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 61 kDa

Lanes 1 - 3: Merged signal (red and green). Green - ab207446 observed at 68 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab207446 was shown to specifically recognize CHEK2 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when CHEK2 knockout samples were examined. Wild-type and CHEK2 knockout samples were subjected to SDS-PAGE. Ab207446 and **ab9484** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Chk2 antibody [EPR19482] (ab207446)

Lanes 1-3 & 5 : Anti-Chk2 antibody [EPR19482] (ab207446) at 1/1000 dilution
Lane 4 : Anti-Chk2 antibody [EPR19482] (ab207446) at 1/5000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 3 : HEK-293T (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 4 : HCT 116 (Human colorectal carcinoma cell line) whole cell lysate
Lane 5 : SH-SY5Y (Human neuroblastoma cell line from bone marrow) whole cell lysate

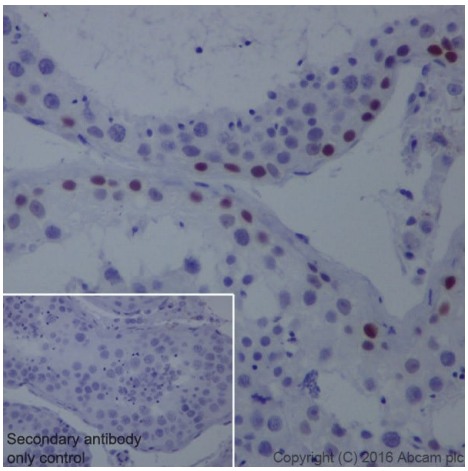
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1/3: 15 seconds; Lane 2: 8 seconds; Lane 4: 30 seconds; Lane 5: 3 minutes.

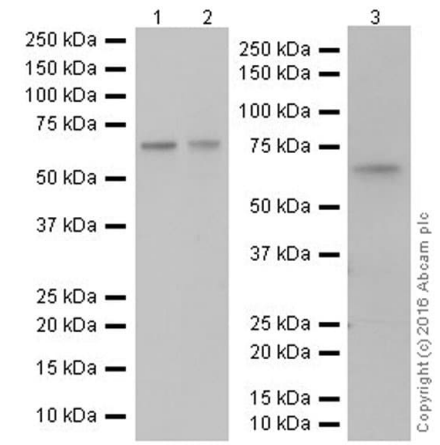


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Chk2 antibody [EPR19482] (ab207446)

Immunohistochemical analysis of paraffin-embedded Human testis tissue labeling Chk2 with ab207446 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on human spermatogonium is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Chk2 antibody [EPR19482] (ab207446)

All lanes : Anti-Chk2 antibody [EPR19482] (ab207446) at 1/1000 dilution

Lane 1 : Human colon lysate
Lane 2 : Human thymus lysate
Lane 3 : Human kidney lysate

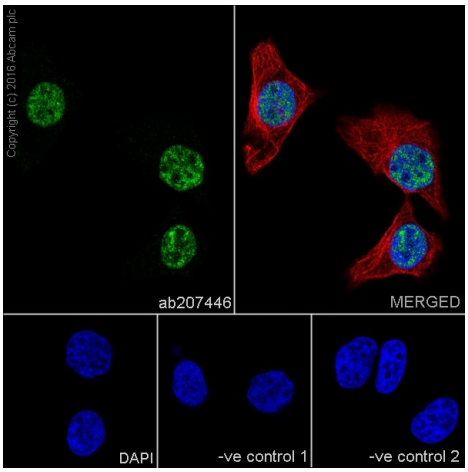
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

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

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Chk2 antibody [EPR19482] (ab207446)

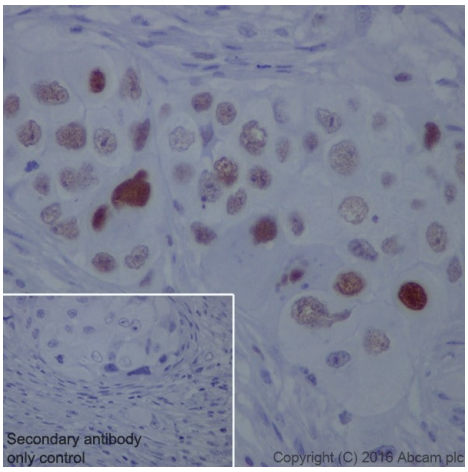
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Chk2 with ab207446 at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab207446 at 1/500 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary at 1/1000 dilution.

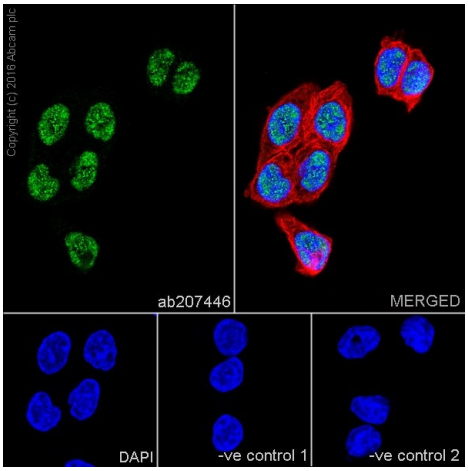


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Chk2 antibody [EPR19482] (ab207446)

Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling Chk2 with ab207446 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on tumor cells of human breast cancer is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Chk2 antibody [EPR19482] (ab207446)

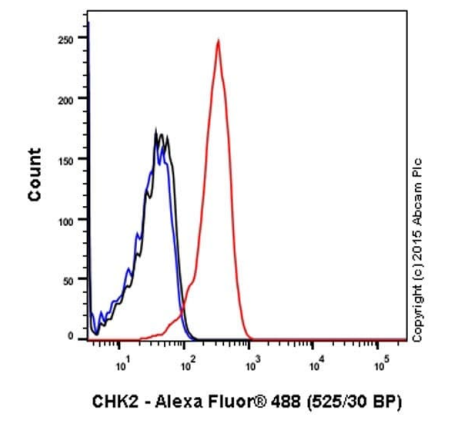
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HCT 116 (Human colorectal carcinoma cell line) cells labeling Chk2 with ab207446 at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HCT 116 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab207446 at 1/500 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary at 1/1000 dilution.

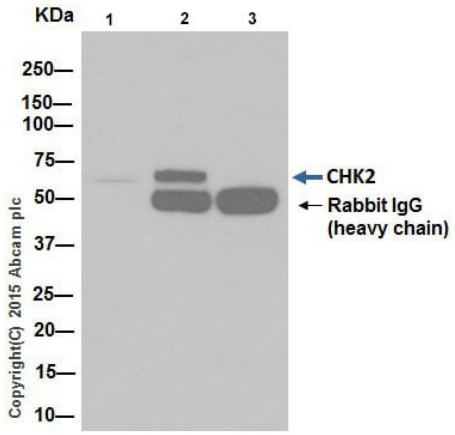
-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Chk2 antibody [EPR19482] (ab207446)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HCT 116 (Human colorectal carcinoma cell line) cells labeling Chk2 with ab207446 at 1/70 dilution (red) compared with a rabbit monoclonal IgG isotype control (**ab172730**; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (Alexa Fluor® 488) at 1/500 dilution was used as the secondary antibody.

Note: Cells were permeabilised with 90% methanol-PBS, -20°C, 30min



Immunoprecipitation - Anti-Chk2 antibody [EPR19482] (ab207446)

Chk2 was immunoprecipitated from 1mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab207446 at 1/40 dilution. Western blot was performed from the immunoprecipitate using ab207446 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

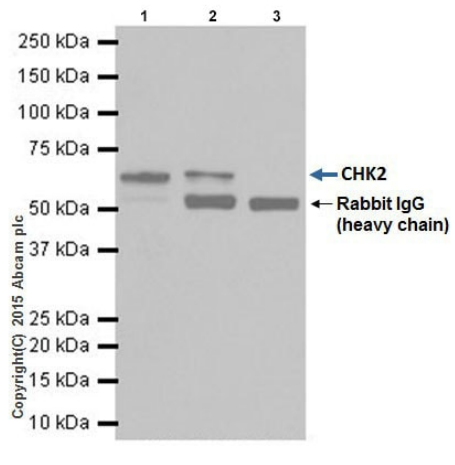
Lane 1: HeLa whole cell lysate 10µg (Input).

Lane 2: ab207446 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab207446 in HeLa whole cell lysate.

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

Exposure time: 10 seconds.



Immunoprecipitation - Anti-Chk2 antibody [EPR19482] (ab207446)

Chk2 was immunoprecipitated from 1mg of HEK-293T (Human epithelial cell line from embryonic kidney) whole cell lysate with ab207446 at 1/40 dilution. Western blot was performed from the immunoprecipitate using ab207446 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

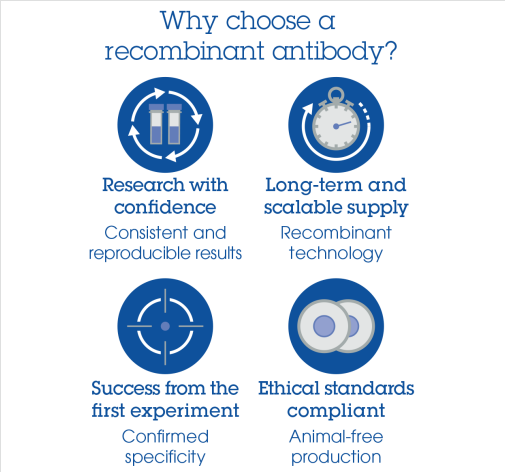
Lane 1: HEK-293T whole cell lysate 10µg (Input).

Lane 2: ab207446 IP in HEK-293T whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab207446 in HEK-293T whole cell lysate.

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

Exposure time: 10 seconds.



Anti-Chk2 antibody [EPR19482] (ab207446)

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