abcam

ab223535

Anti-HP1 gamma/CBX3 antibody [EPR19802] - 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	EPR19802
Purification technique	Affinity purification Protein A
Concentration	0.98 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-

Notes	-

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human, Rat, Mouse
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human, Mouse
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-

Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Mouse, Human, Rat
Dilution info	-
Notes	-

Target data

See full target information CBX3

Function	Component of heterochromatin, which recognizes and binds histone H3 tails methylated at 'Lys-9', leading to epigenetic repression (PubMed:11242053). Also recognizes and binds histone H1.4 methylated at 'Lys-26' (H1.4K26me) (PubMed:16127177). Excluded from chromatin when histone H1.4 is Simultaneously methylated at Lys-26 (H1.4K26me) and phosphorylated at Ser-27 (H1.4S27Ph) (PubMed:16127177). Involved in the formation of functional kinetochore through interaction with MIS12 complex proteins (PubMed:15502821). Contributes to the conversion of local chromatin to a heterochromatin-like repressive state through H3 'Lys-9' trimethylation, mediates the recruitment of the methyltransferases SUV39H1 and/or SUV39H2 by the PER complex to the E-box elements of the circadian target genes such as PER2 itself or PER1 (By similarity). Mediates the recruitment of NIPBL to sites of DNA damage at double-strand breaks (DSBs) (PubMed:28167679).

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

ab223535 is the carrier-free version of ab217999.

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.

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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The HP1 gamma protein also known as CBX3 is an essential component of the chromatin organization machinery. It has a molecular weight of approximately 22-25 kDa. HP1 gamma is expressed in a variety of tissues including brain liver and muscle indicating its broad functional involvement across different biological systems. It exhibits a high affinity for binding to histone H3 when it is methylated facilitating the formation of heterochromatin which is key to regulating gene expression and maintaining genome stability.
Biological function summary	HP1 gamma plays a significant role in gene silencing and chromatin structure maintenance. It forms part of a protein complex that includes chromatin remodeling enzymes and transcriptional repressors which help regulate access to genomic DNA. HP1 gamma's association with other chromatin-associated proteins

	helps modulate the transcriptional response to cellular stimuli ensuring appropriate gene expression patterns needed for normal cell function and development.
Pathways	HP1 gamma is involved in heterochromatin formation and maintenance pathways working alongside other proteins like SUV39H1 and HP1 alpha. Through these pathways HP1 gamma ensures the compact organization of repetitive DNA sequences and suppresses unnecessary transcription. It is also involved in the DNA damage response pathway playing a role in preserving genomic integrity by interacting with proteins such as BRCA1 and 53BP1 involved in DNA repair.
Associated diseases and disorders	HP1 gamma's dysregulation has been associated with cancer and neurological disorders. Overexpression or mutations in this protein can lead to aberrant gene silencing contributing to oncogenesis where cancer-related genes become improperly regulated. Additionally alterations in HP1 gamma function are linked to neurodegenerative diseases potentially through its interaction with proteins like MECP2 whereby changes in the chromatin environment influence neuronal gene expression and function. Therefore understanding HP1 gamma's role provides insights into potential therapeutic targets for these conditions.

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

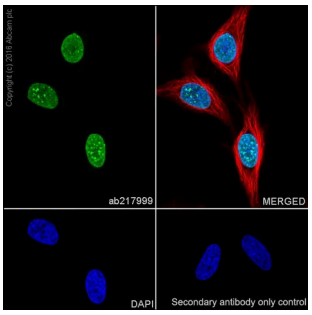
We do not recommend this combination. It is not covered by our product promise.

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.

10 product images



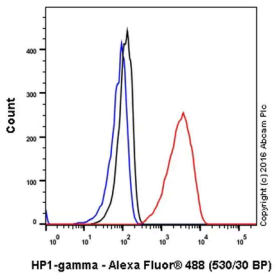
Immunocytochemistry/ Immunofluorescence - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

HP1 gamma/CBX3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HP1 gamma/CBX3 antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling HP1 gamma/CBX3 with ab217999 at 1/1000 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 counterstain is DAPI (blue). Tubulin is detected with ab195889 (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.

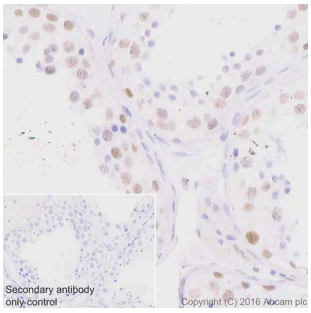
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217999).



Flow Cytometry (Intracellular) - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

HP1 gamma/CBX3 Flow Cytometry (Intracellular) staining using rabbit Anti-HP1 gamma/CBX3 antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling HP1 gamma/CBX3 with ab217999 at 1/400 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217999).



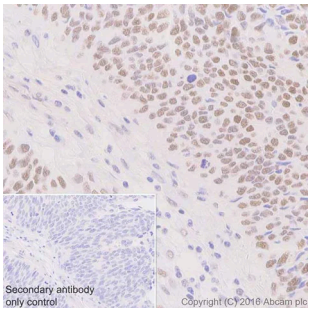
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

HP1 gamma/CBX3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HP1 gamma/CBX3 antibody

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling HP1 gamma/CBX3 with ab217999 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on human testis is observed [PMID: 19786570]. 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217999).

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



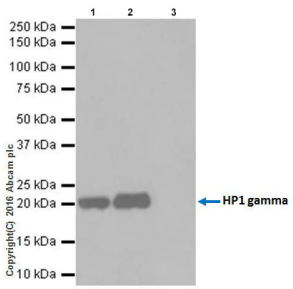
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

HP1 gamma/CBX3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HP1 gamma/CBX3 antibody

Immunohistochemical analysis of paraffin-embedded human bladder cancer tissue labeling HP1 gamma/CBX3 with ab217999 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 bladder 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217999).

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



Immunoprecipitation - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

HP1 gamma/CBX3 was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab217999 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab217999 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, 10 µg (Input).

Lane 2: ab217999 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab217999 in HeLa whole cell lysate.

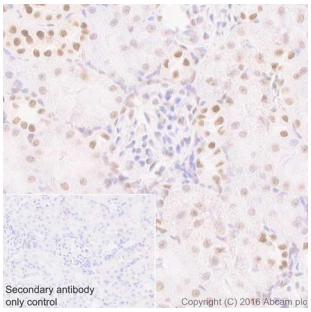
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217999).
All lanes:
Immunoprecipitation - Anti-HP1 gamma/CBX3 antibody [EPR19802] (ab217999)

Predicted band size: 20 kDa

Observed band size: 22 kDa



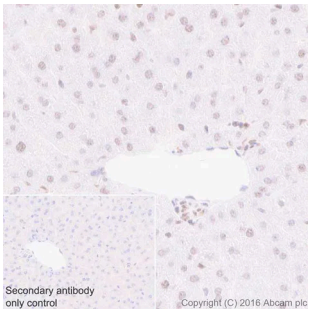
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

HP1 gamma/CBX3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HP1 gamma/CBX3 antibody

Immunohistochemical analysis of paraffin-embedded rat kidney tissue labeling HP1 gamma/CBX3 with ab217999 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on rat kidney 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217999).

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



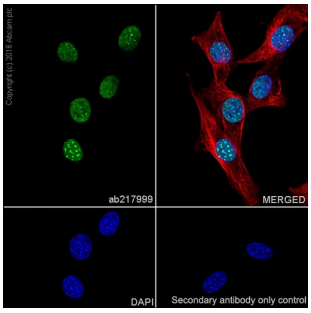
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

HP1 gamma/CBX3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-HP1 gamma/CBX3 antibody

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling HP1 gamma/CBX3 with ab217999 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on hepatocytes of mouse liver 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217999).

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



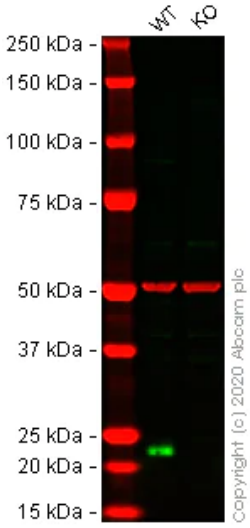
Immunocytochemistry/ Immunofluorescence - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

HP1 gamma/CBX3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-HP1 gamma/CBX3 antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling HP1 gamma/CBX3 with ab217999 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on NIH/3T3 cell line.
The nuclear counterstain is DAPI (blue). Tubulin is detected with ab195889 (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab217999).



Western blot - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

HP1 gamma/CBX3 Western blot staining using rabbit Anti-HP1 gamma/CBX3 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab217999).
Lanes 1- 2: Merged signal (red and green). Green - ab217999 observed at 25 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) observed at 50 kDa.

ab217999 was shown to react with HP1 gamma/CBX3 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab261744 (knockout cell lysate ab257110) was used. Wild-type HeLa and CBX3 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab217999 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) overnight at 4°C at a 1 in 2000 dilution and a 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-HP1 gamma/CBX3 antibody [EPR19802] (ab217999) at 1/2000 dilution

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

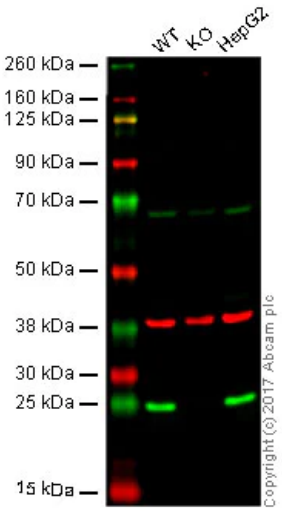
Lane 2:
CBX3 knockout HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human CBX3 (HP1 gamma) knockout HeLa cell line (ab261744)

Performed under reducing conditions.

Predicted band size: 20 kDa

Observed band size: 25 kDa



Western blot - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

HP1 gamma/CBX3 Western blot staining using rabbit Anti-HP1 gamma/CBX3 antibody

This WB data was generated using the same anti-HP1 gamma/CBX3 antibody clone [EPR19802] in a different buffer format (cat# ab217999).
Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: CBX3 knockout HAP1 whole cell lysate (20 µg)
Lane 3: HepG2 whole cell lysate (20 µg)

Lanes 1 - 3: Merged signal (red and green). Green - ab217999 observed at 25 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab217999 was shown to recognize CBX3 when CBX3 knockout samples were used, along with additional cross-reactive bands. Wild-type and CBX3 knockout samples were subjected to SDS-PAGE. ab217999 and ab9484 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 2000 dilution and 1/10000 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.

All lanes:
Western blot - Anti-HP1 gamma/CBX3 antibody [EPR19802] (ab217999)

Predicted band size: 20 kDa

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