

Product datasheet

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

KO VALIDATED Recombinant RabMAb

11 Images

Overview

Product name	Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free
Description	Rabbit monoclonal [EPR19802] to HP1 gamma/CBX3 - BSA and Azide free
Host species	Rabbit
Tested applications	Suitable for: ICC/IF, IP, Flow Cyt, WB, IHC-P
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide aa 100 to the C-terminus. The exact sequence is proprietary. Database link: P23198
Positive control	WB: Human brain lysate; HeLa, HepG2, PC-3, C2C12, 4T1, A549, LNCaP, C6 and NIH/3T3 whole cell lysates; Mouse and rat brain lysates. IHC-P: Human testis and bladder cancer tissues; Mouse liver tissue; Rat kidney tissue. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt: HeLa cells. IP: HeLa whole cell lysate.
General notes	<p>ab223535 is the carrier-free version of ab217999 This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes.</p> <p>Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency.</p> <p>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.</p> <p>Ab223535 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm.</p> <p><i>Maxpar® is a trademark of Fluidigm Canada Inc.</i></p> <p>This product was previously labelled as HP1 gamma</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p>

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to [RabMAb[®] patents](#).

Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise[™] guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19802
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab223535** 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
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

Application	Abreviews	Notes
Flow Cyt		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 21 kDa (predicted molecular weight: 21 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

Function

Seems to be involved in transcriptional silencing in heterochromatin-like complexes. Recognizes and binds histone H3 tails methylated at 'Lys-9', leading to epigenetic repression. May contribute to the association of the heterochromatin with the inner nuclear membrane through its interaction with lamin B receptor (LBR). Involved in the formation of functional kinetochore through interaction with MIS12 complex proteins.

Sequence similarities

Contains 2 chromo domains.

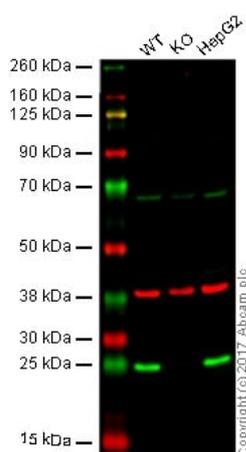
Post-translational modifications

Phosphorylated by PIM1. Phosphorylated during interphase and possibly hyper-phosphorylated during mitosis.

Cellular localization

Nucleus. Associates with euchromatin and is largely excluded from constitutive heterochromatin. May be associated with microtubules and mitotic poles during mitosis.

Images



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

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)

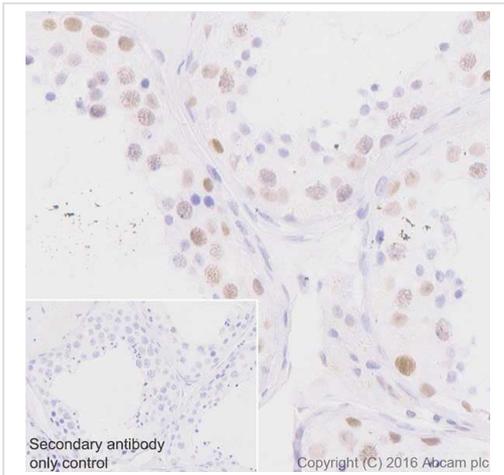
Lane 2: CBX3 knockout HAP1 whole cell lysate (20 µg)

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.



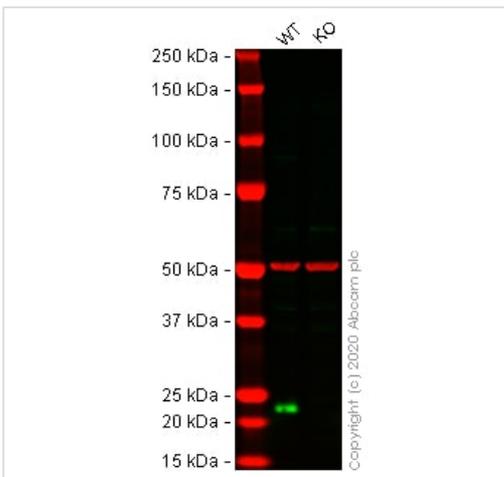
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

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.



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

All lanes : Anti-HP1 gamma/CBX3 antibody [EPR19802] ([ab217999](#)) at 1/2000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : CBX3 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 21 kDa

Observed band size: 25 kDa

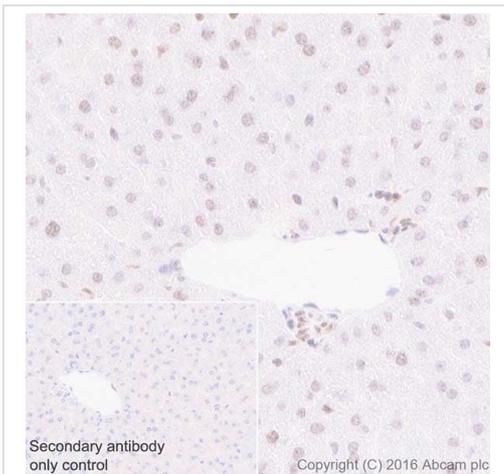
[why is the actual band size different from the predicted?](#)

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.



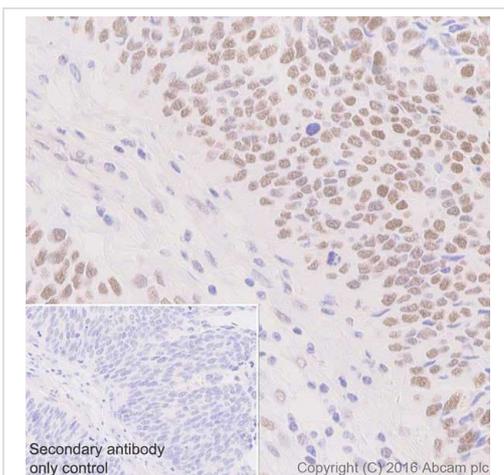
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free ([ab223535](#))

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.



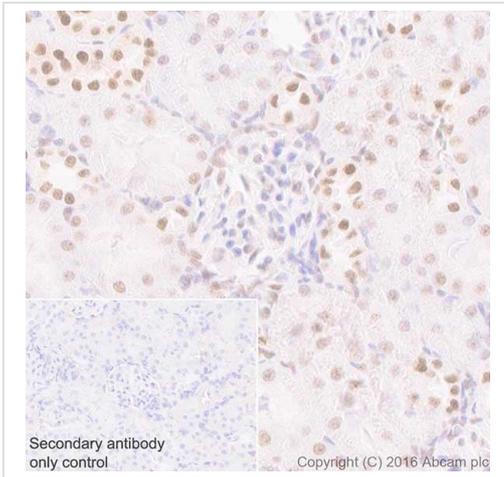
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free ([ab223535](#))

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.



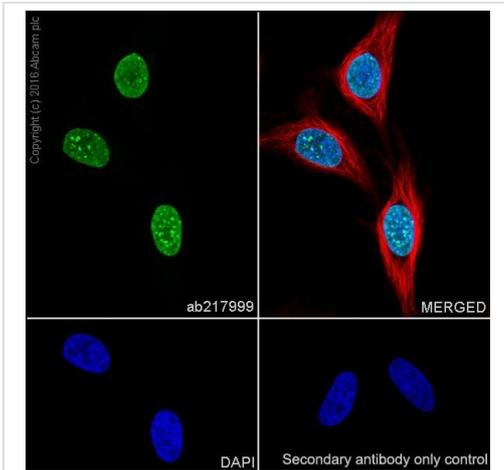
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

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.



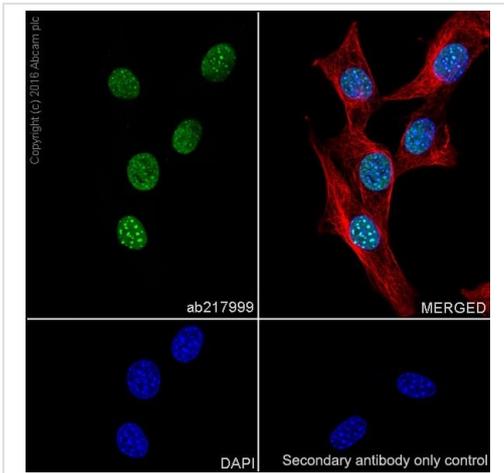
Immunocytochemistry/ Immunofluorescence - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

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](#)).



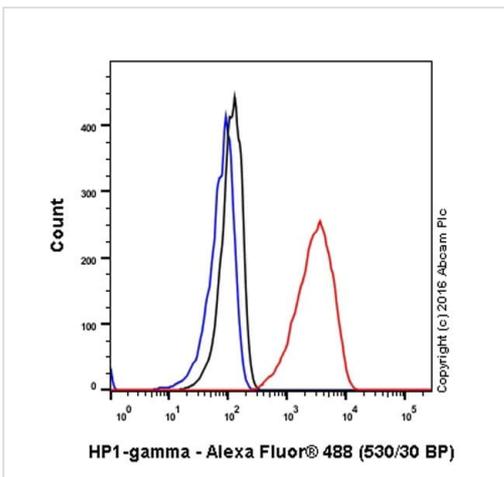
Immunocytochemistry/ Immunofluorescence - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

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.

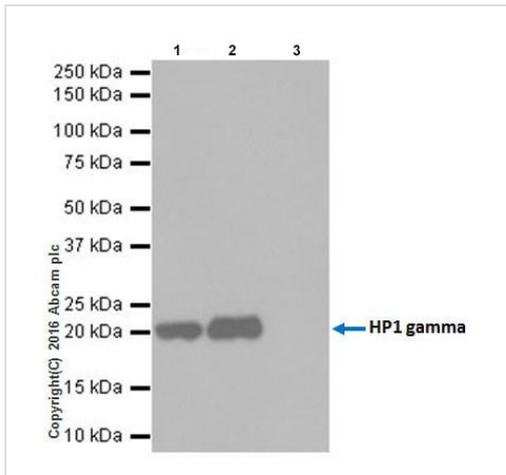
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab217999](#)).



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

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](#)).



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](#)).

Why choose a recombinant antibody?

Research with confidence
Consistent and reproducible results

Long-term and scalable supply
Recombinant technology

Success from the first experiment
Confirmed specificity

Ethical standards compliant
Animal-free production

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

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.com/abpromise> or contact our technical team.

Terms and conditions

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