abcam

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:** Flow Cyt (Intra), ICC/IF, IP, WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
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 (intra): HeLa cells. IP: HeLa whole cell lysate.
General notes	ab223535 is the carrier-free version of **ab217999**. 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. 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. 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. 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. 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

The Abpromise guarantee 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.

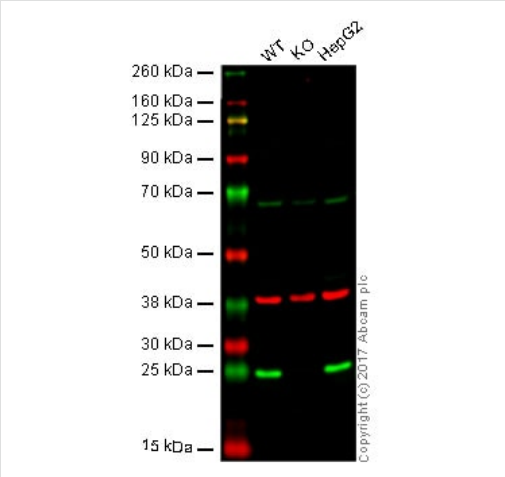
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay 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	Phosphorylated by PIM1. Phosphorylated during interphase and possibly hyper-phosphorylated

modifications	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



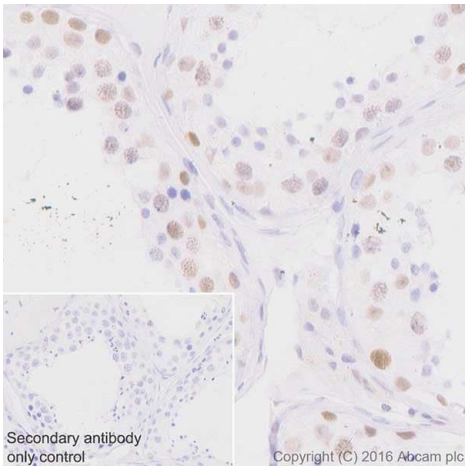
Western blot - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

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.



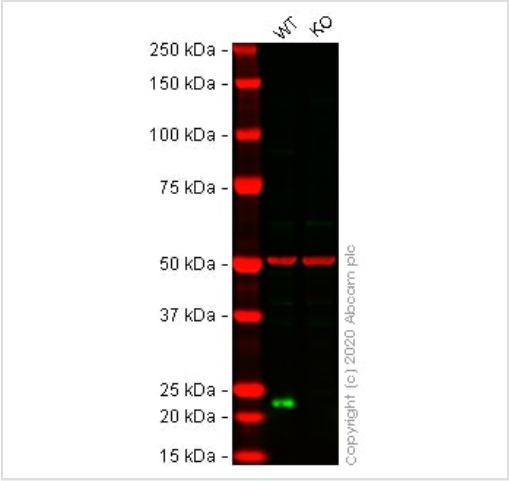
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

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.

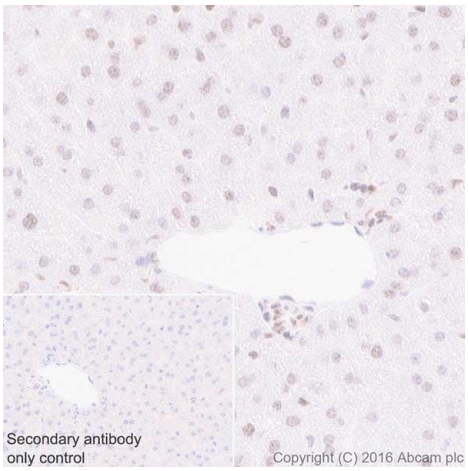
Performed under reducing conditions.

Predicted band size: 21 kDa
Observed band size: 25 kDa

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.



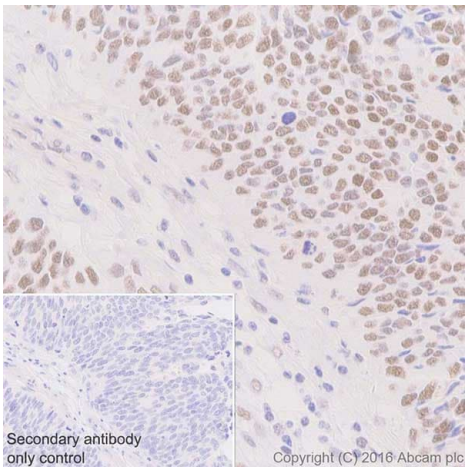
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

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.



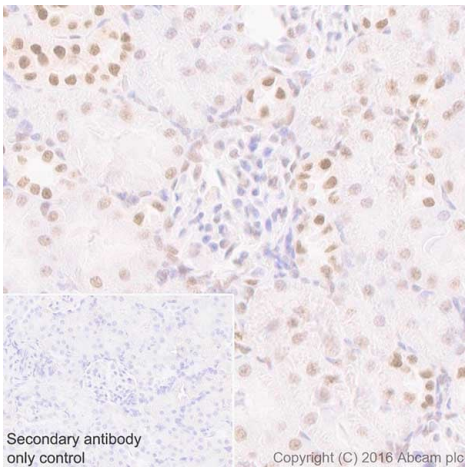
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

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.



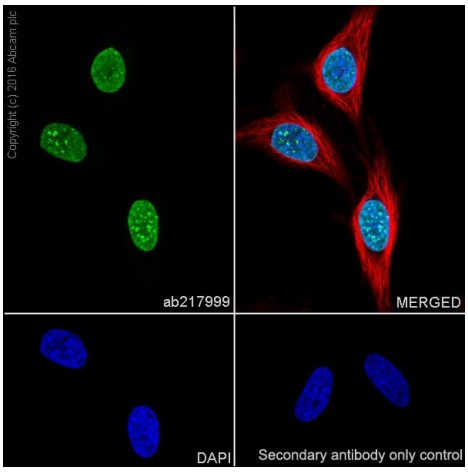
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

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.



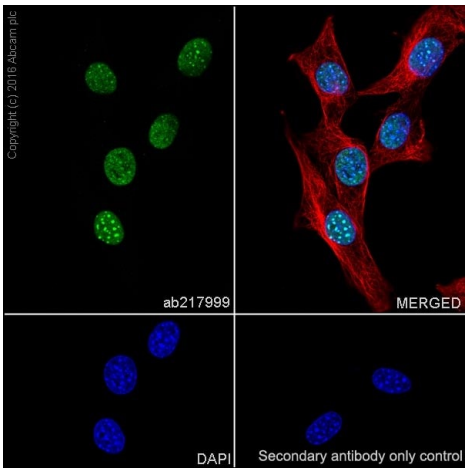
Immunocytochemistry/ Immunofluorescence - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

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**).



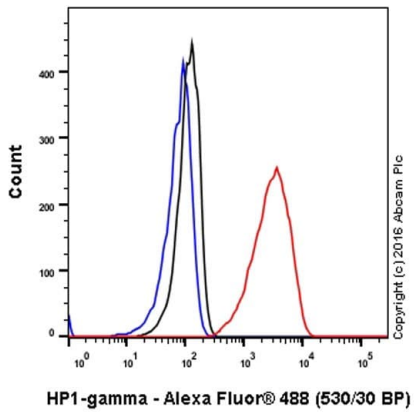
Immunocytochemistry/ Immunofluorescence - Anti-HP1 gamma/CBX3 antibody [EPR19802] - BSA and Azide free (ab223535)

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.

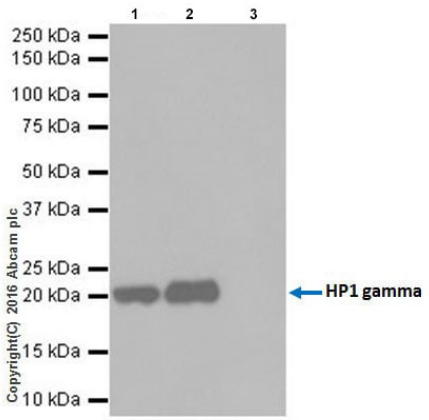
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab217999**).



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

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**).



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).

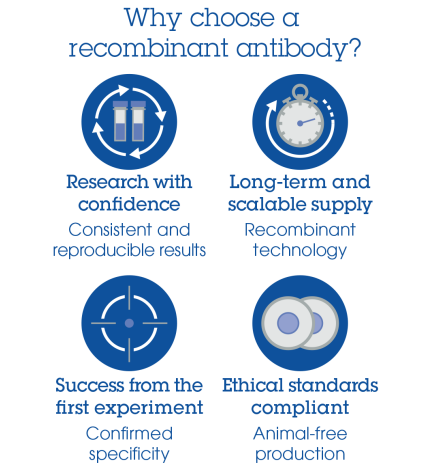
Lane 2: **ab217999** IP in HeLa whole cell lysate.

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**).



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

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