abcam

Product datasheet

Anti-HP1 gamma/CBX3 antibody [EPR19802] ab217999





RabMAb

4 References 14 图像

概述

产品名称 Anti-HP1 gamma/CBX3抗体[EPR19802]

描述 兔单克隆抗体[EPR19802] to HP1 gamma/CBX3

宿主 Rabbit

经测试应用 适用于: WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra)

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 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.

常规说明 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**.

性能

形式 Liquid

存放说明 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.

存储溶液 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

纯**度** Protein A purified

1

同种型 IgG

应用

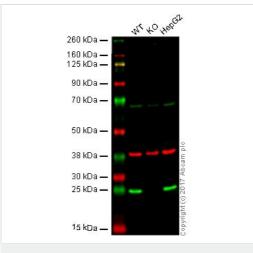
图片

The Abpromise guarantee Abpromise™承诺保证使用ab217999于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

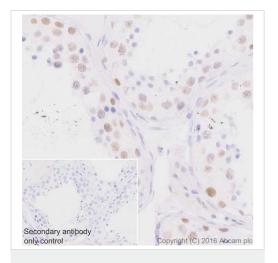
应用	Ab评论	说明
WB		1/2000. Detects a band of approximately 21 kDa (predicted molecular weight: 21 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/1000.
IP		1/30.
Flow Cyt (Intra)		1/400. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

功能	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.
序列相似性	Contains 2 chromo domains.
翻译后修饰	Phosphorylated by PIM1. Phosphorylated during interphase and possibly hyper-phosphorylated during mitosis.
细胞定位	Nucleus. Associates with euchromatin and is largely excluded from constitutive heterochromatin. May be associated with microtubules and mitotic poles during mitosis.



[EPR19802] (ab217999)

Western blot - Anti-HP1 gamma/CBX3 antibody



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] (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)

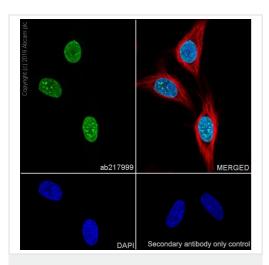
Lanes 1 - 3: Merged signal (red and green). Green - ab217999 observed at 25 kDa. Red - loading control, ab9484, observed at 37 kDa.

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 lgG 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.

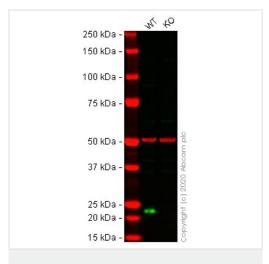
Immunohistochemical analysis of paraffin-embedded human testis tissue labeling HP1 gamma/CBX3 with ab217999 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on human testis is observed [PMID: 19786570]. Counter stained with Hematoxylin.

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.

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] (ab217999)



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

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 lgG (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 lgG (Alexa Fluor[®] 488) (ab150077) at 1/1000 dilution.

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

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.

1 2 3 4 5 6 250 kDa -150 kDa -100 kDa -75 kDa -50 kDa -37 kDa -Copyright (c) 2016 Abcam plo 25 kDa 🕳 20 kDa 🕳 15 kDa -10 kDa 🕳 Western blot - Anti-HP1 gamma/CBX3 antibody

[EPR19802] (ab217999)

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

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lane 3 : PC-3 (Human prostate adenocarcinoma cell line) whole cell lysate

Lane 4 : C2C12 (Mouse myoblast cell line) whole cell lysate

Lane 5: 4T1 (Mouse mammary gland carcinoma cell line) whole cell lysate

Lane 6 : A549 (Human lung carcinoma cell line) whole cell lysate

Lane 7 : LNCaP (Human prostate cancer cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

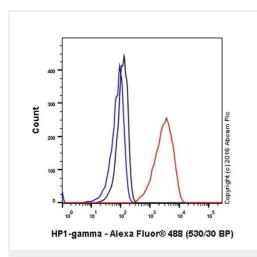
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 21 kDa **Observed band size:** 21 kDa

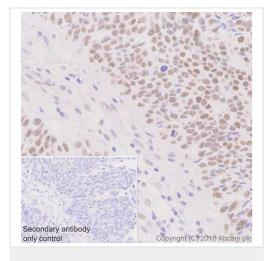
Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1,2,3,4 and 5: 1 second; Lane 6 and 7: 3

seconds.



Flow Cytometry (Intracellular) - Anti-HP1 gamma/CBX3 antibody [EPR19802] (ab217999) Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling HP1 gamma/CBX3with ab217999 at 1/400 dilution (red) compared with a rabbit monoclonal lgG isotype control (ab172730; black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit lgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.

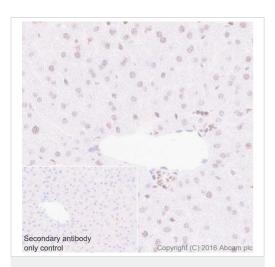


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] (ab217999)

Immunohistochemical analysis of paraffin-embedded human bladder cancer tissue labeling HP1 gamma/CBX3 with ab217999 at 1/2000 dilution, followed by Goat Anti-Rabbit lgG 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 lgG 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.

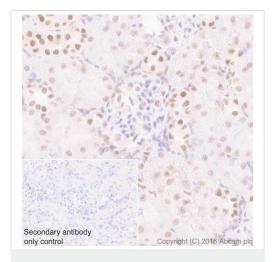


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] (ab217999)

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 lgG 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.

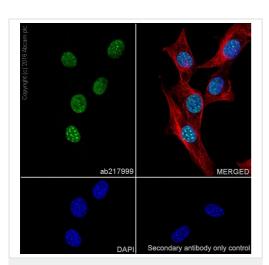


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HP1 gamma/CBX3 antibody [EPR19802] (ab217999)

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 lgG 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.

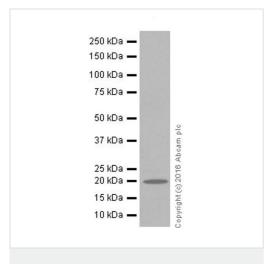


Immunocytochemistry/ Immunofluorescence - Anti-HP1 gamma/CBX3 antibody [EPR19802] (ab217999)

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 lgG (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 lgG (Alexa Fluor[®] 488) (ab150077) at 1/1000 dilution.



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

Anti-HP1 gamma/CBX3 antibody [EPR19802] (ab217999) at 1/2000 dilution + Human brain lysate at 10 µg

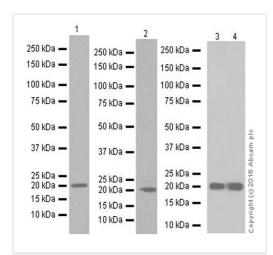
Secondary

Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/4000 dilution

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

Exposure time: 3 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



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

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

Lane 1: Mouse brain lysate

Lane 2: Rat brain lysate

Lane 3: C6 (Rat glial tumor cell line) whole cell lysate

Lane 4: NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell

lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at

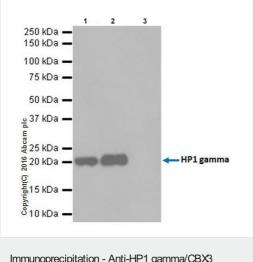
1/100000 dilution

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

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 3 seconds; Lane 2: 10 seconds; Lane 3

and 4: 3 seconds.



Immunoprecipitation - Anti-HP1 gamma/CBX3 antibody [EPR19802] (ab217999)

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.

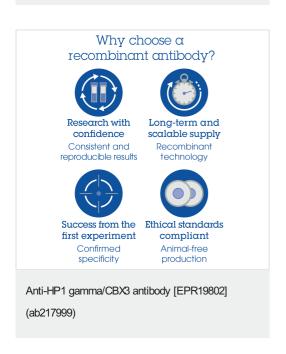
Lane 1: HeLa whole cell lysate, 10 µg (Input).

Lane 2: ab217999 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of ab217999 in HeLa whole cell lysate.

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

Exposure time: 1 second.



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.cn/abpromise or contact our technical team.

Terms and conditions

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