# abcam

## Product datasheet

## Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker ab109028





重组 RabMAb

★★★★★ 8 Abreviews 13 References 10 图像

#### 概述

产品名称 Anti-HP1 alpha抗体[EPR5777] - Heterochromatin marker

描述 兔单克隆抗体[EPR5777] to HP1 alpha - Heterochromatin marker

宿主 Rabbit

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

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 ICC/IF: HAP1 and HeLa cells; IP: MCF7 cell lysate; Flow Cyt (intra): HeLa cells; IHC-P: Human

breast cancer, rat pancreas, and mouse kidney tissue sections; WB: HeLa, SH-SY5Y whole cell

lysates, Rat and mouse brain lysates.

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: 7.20

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS

纯度 Protein A purified

克隆 单克隆

**克隆编号** EPR5777

**同种型** IgG

#### 应用

## The Abpromise guarantee Abpromise™承诺保证使用ab109028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                                                                                                                                                                        |
|------------------|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Flow Cyt (Intra) |                  | 1/10 - 1/100. <b>ab172730</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.                                                      |
| ICC/IF           | <b>★★★★★ (4)</b> | 1/250.                                                                                                                                                                    |
| WB               | <b>★★★★★</b> (4) | 1/1000. Detects a band of approximately 22 kDa (predicted molecular weight: 22 kDa).  For unpurified use at 1/10000 - 1/50000.                                            |
| IP               |                  | 1/10 - 1/100.                                                                                                                                                             |
| IHC-P            |                  | 1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.  See IHC antigen retrieval protocols.  For unpurified use at 1/250 - 1/500. |

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功能 Component of heterochromatin that recognizes and binds histone H3 tails methylated at 'Lys-9'

(H3K9me), leading to epigenetic repression. In contrast, it is excluded from chromatin when 'Tyr-41' of histone H3 is phosphorylated (H3Y41ph). Can interact with lamin-B receptor (LBR). This interaction can contribute to the association of the heterochromatin with the inner nuclear membrane. 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 Phosphorylation of HP1 and LBR may be responsible for some of the alterations in chromatin

organization and nuclear structure which occur at various times during the cell cycle (By similarity).

Phosphorylated during interphase and possibly hyper-phosphorylated during mitosis.

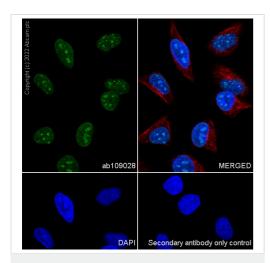
Ubiquitinated.

细胞定位 Nucleus. Chromosome. Chromosome > centromere. Component of centromeric and

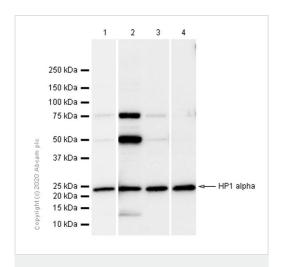
pericentromeric heterochromatin. Associates with chromosomes during mitosis. Associates

specifically with chromatin during metaphase and anaphase.

图片



Immunocytochemistry/ Immunofluorescence - Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker (ab109028)



Western blot - Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker (ab109028)

ab109028 staining HP1 alpha in HeLa (human cervical adenocarcinoma epithelial cell). The cells were fixed with 100% methanol, permeabilized with 0.1% TritonX-100. The cells were then incubated with ab109028 at 1:100 dilution. Cells were then incubated with ab150088, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed at 1:1000 dilution (shown in green).

ab195889, Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used as counterstain at 1:200 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Confocal image showing nuclear staining in HeLa cell line.

**All lanes :** Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker (ab109028) at 1/1000 dilution (Purified)

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

**Lane 2**: SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysate

Lane 3: Mouse brain lysate

Lane 4: Rat brain lysate

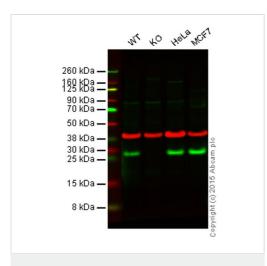
Lysates/proteins at 20 µg per lane.

### **Secondary**

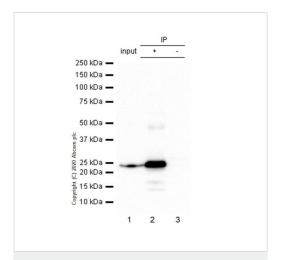
**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 22 kDa

We are unsure how to define the extra bands.



Western blot - Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker (ab109028)



Immunoprecipitation - Anti-HP1 alpha antibody
[EPR5777] - Heterochromatin marker (ab109028)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: HP1 alpha knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: MCF7 cell lysate (20 µg)

**Lanes 1 - 4**: Merged signal (red and green). Green - ab109028 observed at 28 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab109028 was shown to specifically react with HP1 alpha when HP1 alpha knockout samples were used. Wild-type and HP1 alpha knockout samples were subjected to SDS-PAGE. ab109028 and <u>ab8245</u> (loading control to GAPDH) were diluted 1/1000 and 1/20 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.

Purified ab109028 at 1/30 dilution ( $2\mu g$ ) immunoprecipitating HP1 alpha in MCF7 whole cell lysate.

Lane 1 (input): MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate  $10\mu g$ 

Lane 2 (+): ab109028 + MCF7 whole cell lysate.

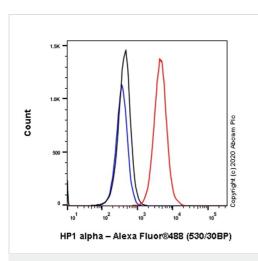
Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab109028 in MCF7 whole cell lysate.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (1/1000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

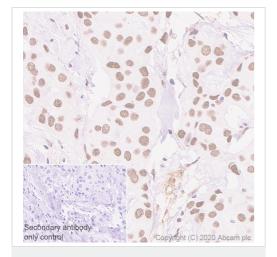
Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 22 kDa



Flow Cytometry (Intracellular) - Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker (ab109028)

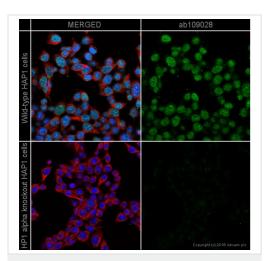
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling HP1 alpha with Purified ab109028 at 1/30 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor<sup>®</sup> 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HP1 alpha antibody

[EPR5777] - Heterochromatin marker (ab109028)

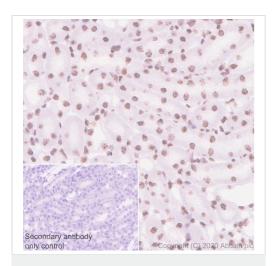
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast cancer tissue sections labeling HP1 alpha with Purified ab109028 at 1/1000 dilution (0.31 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunocytochemistry/ Immunofluorescence - Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker (ab109028)

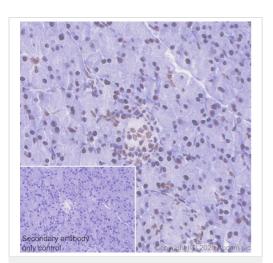
ab109028 staining HP1 alpha in wild-type HAP1 cells (top panel) and HP1 alpha knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab109028 at 1/250 dilution and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit lgG (Alexa Fluor® 488) (ab150081) at 2  $\mu$ g/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HP1 alpha antibody
[EPR5777] - Heterochromatin marker (ab109028)

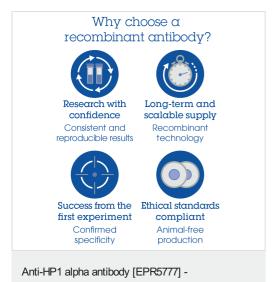
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue sections labeling HP1 alpha with Purified ab109028 at 1/1000 dilution (0.31 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HP1 alpha antibody

[EPR5777] - Heterochromatin marker (ab109028)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat pancreas tissue sections labeling HP1 alpha with Purified ab109028 at 1/1000 dilution (0.31 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Heterochromatin marker (ab109028)

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