abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

★★★★★ 8 Abreviews 8 References 10 Images

Overview

Product name	Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker
Description	Rabbit monoclonal [EPR5777] to HP1 alpha - Heterochromatin marker
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC, WB, IP, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	ICC: 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.
General notes	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. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.**

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5777
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab109028 in the following tested applications.

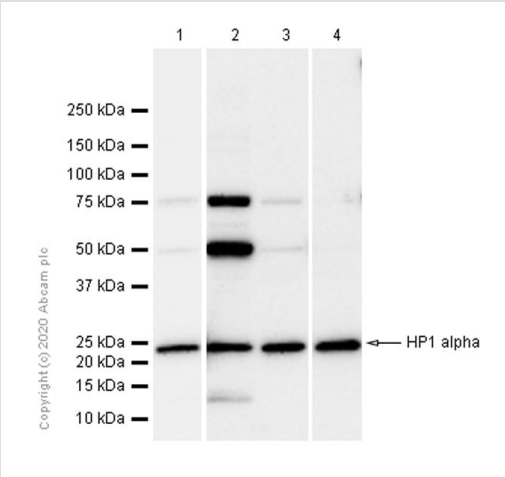
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/10 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC		1/250.
WB	★★★★★ (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.**

Target

Function	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.
Sequence similarities	Contains 2 chromo domains.
Post-translational modifications	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.
Cellular localization	Nucleus. Chromosome. Chromosome > centromere. Component of centromeric and pericentromeric heterochromatin. Associates with chromosomes during mitosis. Associates specifically with chromatin during metaphase and anaphase.

Images



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

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

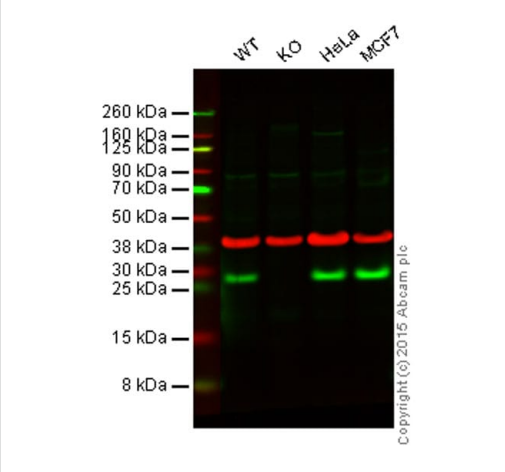
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysate
Lane 3 : Mouse brain lysate
Lane 4 : Rat brain lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 22 kDa

We are unsure how to define the extra bands.

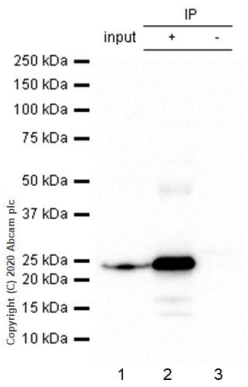


Western blot - Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker (ab109028)

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)

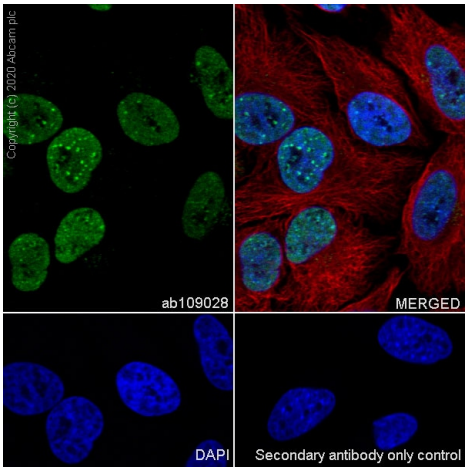
Lanes 1 - 4: Merged signal (red and green). Green - ab109028 observed at 28 kDa. Red - loading control, ab8245, observed at 37 kDa.

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 ab8245 (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 (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



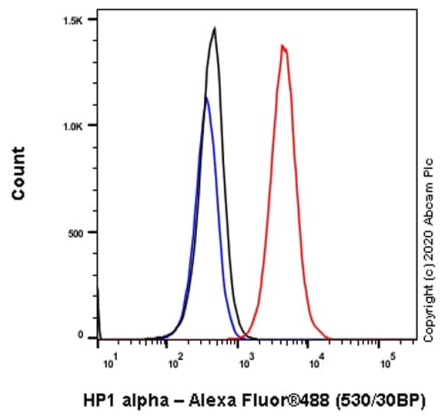
Immunoprecipitation - Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker (ab109028)

Purified ab109028 at 1/30 dilution (2µg) immunoprecipitating HP1 alpha in MCF7 whole cell lysate.
Lane 1 (input): MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab109028 + MCF7 whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab109028 in MCF7 whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (ab131366) (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



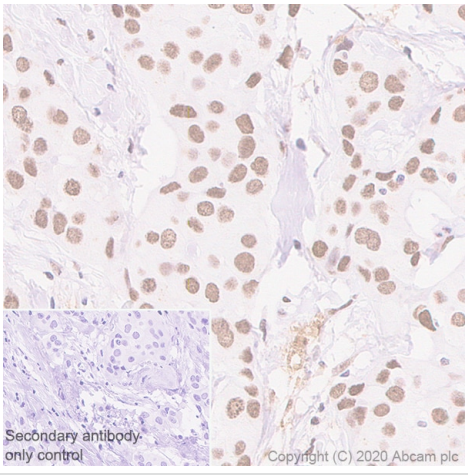
Immunocytochemistry - Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker (ab109028)

Immunocytochemistry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling HP1 alpha with Purified ab109028 at 1/250 dilution (1.22 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 dilution (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 dilution (2 µg/mL). DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



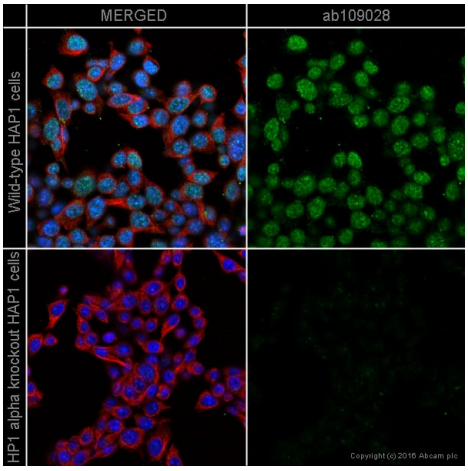
Flow Cytometry (Intracellular) - Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker (ab109028)

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® 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 paraffin-embedded sections) - Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker (ab109028)

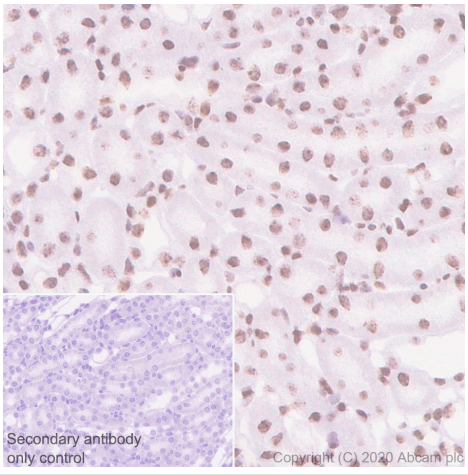
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast cancer tissue sections labeling HP1 alpha with Purified ab109028 at 1/1000 dilution (0.31 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer 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 - Anti-HP1 alpha antibody [EPR5777] - Heterochromatin marker (ab109028)

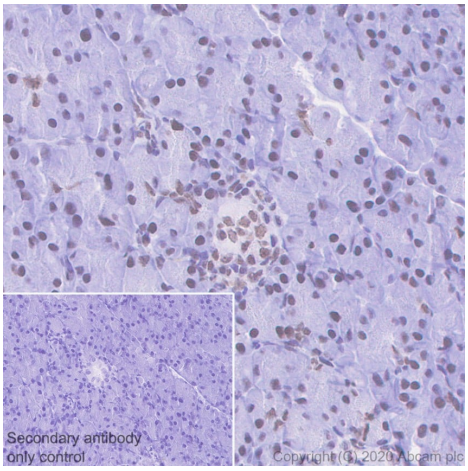
ab109028 staining HP1 alpha in wild-type HAP1 cells (top panel) and HP1 alpha knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 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 IgG (Alexa Fluor® 488) (ab150081) at 2 µg/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 paraffin-embedded 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 paraffin-embedded 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.

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

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