abcam

ab251364

Anti-HP1 gamma/CBX3 (citrulline R108) antibody [EPR19802-202] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR19802-202
Purification technique	Affinity purification Protein A
Specificity	Treatment is required for detection of the target protein. See Protocol section for detailed information. Protocol .

Reactivity data

IP

Tested

Species	Human
Dilution info	-

Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

Not recommended

Species	Transfected cell lysate
Dilution info	-
Notes	-

Dot

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

Not recommended

Species	Transfected cell lysate
Dilution info	-
Notes	-

WB

Tested

Species	Transfected cell lysate, Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

Not recommended

Species	Transfected cell lysate
Dilution info	-
Notes	-

Target data

See full target information Cbx3 citR108

Function	Component of heterochromatin, which recognizes and binds histone H3 tails methylated at 'Lys-9', leading to epigenetic repression (By similarity). Also

recognizes and binds histone H1.4 methylated at 'Lys-26' (H1.4K26me) (By similarity). Involved in the formation of functional kinetochore through interaction with MIS12 complex proteins (By similarity). Involved in the formation of functional kinetochore through interaction with MIS12 complex proteins (By similarity). Contributes to the conversion of local chromatin to a heterochromatin-like repressive state through H3 'Lys-9' trimethylation, mediates the recruitment of the methyltransferases SUV39H1 and/or SUV39H2 by the PER complex to the E-box elements of the circadian target genes such as PER2 itself or PER1 (PubMed:24413057). Mediates the recruitment of NIPBL to sites of DNA damage at double-strand breaks (DSBs) (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab251364 is the carrier-free version of ab202107.

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Treatment Conditions:

Chemical treatment is required for detection of endogenous citrullinated proteins in cell lines. PADs (including PADI2 and PADI4) are the enzymes that hydrolyze arginine to citrulline, in the presence of Ca^{2+} and ionomycin. Please refer to PMID: 26360112

Controls:
- Mock (no transfection)
- Transfection reagent–only control
- Empty vector control
- Vehicle control for chemical treatment

Generation of PADI2 or PADI4 overexpression cell line and treatment with $CaCl_2$/Ionomycin:

1. Prior to transfection with PADI2 or PADI4 expression vector, seed cells in complete medium and place in a 37°C, 5% CO_2 incubator for 24 hours or until cells reach 70-80% confluence.

For a single T-175 flask we recommend seeding the following common cell lines as follows in 30 mL media:
- HEK-293T: $1x10^7$ cells
- MCF7: $2.5\text{-}4x10^6$ cells

2. Once cells have reached confluence, prepare the PADI2 or PADI4 expression vector for transfection using your preferred method (PMID: 33976969).
3. 24-48 hours after transfection, remove the medium from the cells and replace with fresh complete medium containing 10 mM $CaCl_2$ (ab285384) and 10 uM Ionomycin (ab120116) and incubate for 2-4 hours.

Key Considerations:
For alternative cell lines, you should confirm the transfectability, growth rate, and sensitivity of the cell line to the planned chemical treatment. Incubation times and treatment length may need to be adjusted.

Western Blot Conditions:
Recommended SDS-PAGE Gel Type: 4-20% Tris-glycine
Recommended Running Buffer: Tris-Glycine-SDS
Primary: 1/1000 dilution at 4°C overnight or room temperature for 1 hour
Blocking: 5% NFDM at room temperature for 1 hour

Positive Control: Cell line overexpressing PADI4 and treated with $CaCl_2$/Ionomycin
Negative Control: Cell line overexpressing PADI4

Additional resources:
Lysate preparation protocol
Lysate preparation protocol - nuclear proteins
Lysate preparation protocol - low molecular weight proteins
Western blot troubleshooting

Flow Cytometry Conditions:
Primary: 1/3000 dilution at 4°C for 30 minutes
Fixation: 4% PFA at room temperature for 10 minutes

Permeabilization:

Positive Control: Cell line overexpressing PADI4 and treated with CaCl2/Ionomycin
Negative Control: Cell line overexpressing PADI4
Secondary only control:

Additional resources:
Flow Cytometry protocol
Flow cytometry troubleshooting

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The HP1 gamma protein also known as CBX3 is an essential component of the chromatin organization machinery. It has a molecular weight of approximately 22-25 kDa. HP1 gamma is expressed in a variety of tissues including brain liver and muscle indicating its broad functional involvement across different biological systems. It exhibits a high affinity for binding to histone H3 when it is methylated facilitating the formation of heterochromatin which is key to regulating gene expression and maintaining genome stability.
Biological function summary	HP1 gamma plays a significant role in gene silencing and chromatin structure maintenance. It forms part of a protein complex that includes chromatin remodeling enzymes and transcriptional repressors which help regulate access to genomic DNA. HP1 gamma's association with other chromatin-associated proteins helps modulate the transcriptional response to cellular stimuli ensuring appropriate gene expression patterns needed for normal cell function and development.
Pathways	HP1 gamma is involved in heterochromatin formation and maintenance pathways working alongside other proteins like SUV39H1 and HP1 alpha. Through these pathways HP1 gamma ensures the compact organization of repetitive DNA sequences and suppresses unnecessary transcription. It is also involved in the DNA damage response pathway playing a role in preserving genomic integrity by interacting with proteins such as BRCA1 and 53BP1 involved in DNA repair.
Associated diseases and disorders	HP1 gamma's dysregulation has been associated with cancer and neurological disorders. Overexpression or mutations in this protein can lead to aberrant gene silencing contributing to oncogenesis where cancer-related genes become improperly regulated. Additionally alterations in HP1 gamma function are linked to neurodegenerative diseases potentially through its interaction with proteins like MECP2 whereby changes in the chromatin environment influence neuronal gene expression and function. Therefore understanding HP1 gamma's role provides insights into potential therapeutic targets for these conditions.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

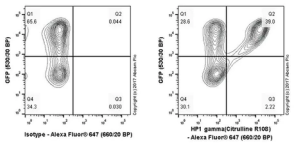
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In the unlikely event of one of our products not working as expected, you are covered by our product promise.

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5 product images

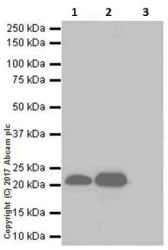


Flow Cytometry (Intracellular) - Anti-HP1 gamma/CBX3 (citrulline R108) antibody [EPR19802-202] - BSA and Azide free (ab251364)

HP1 gamma/CBX3 (citrulline R108) Flow Cytometry (Intracellular) staining using rabbit Anti-HP1 gamma/CBX3 (citrulline R108) antibody

This data was developed using ab202107, the same antibody clone in a different buffer formulation.
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HEK-293 (human epithelial cell line from embryonic kidney) cells transfected with GFP-tagged PADI4 (WT) treated with 10 mM CaCl2 and 10 µM Ionomycin labeling HP1 gamma/CBX3 (citrulline R108) with ab202107 at 1/3000 dilution (right panel) compared with a rabbit monoclonal IgG isotype control (ab172730) (left panel). Goat Anti-Rabbit IgG H+L (Alexa Fluor® 647) (ab150079) was used as the secondary antibody.

Only CaCl2 and Ionomycin treated, GFP positive cell population result in a positive signal (right panel, Q2).



Immunoprecipitation - Anti-HP1 gamma/CBX3 (citrulline R108) antibody [EPR19802-202] - BSA and Azide free (ab251364)

This data was developed using ab202107, the same antibody clone in a different buffer formulation.
HP1 gamma/CBX3 (citrulline R108) was immunoprecipitated from 0.35 mg HEK-293 (human epithelial cell line from embryonic kidney) transfected with GFP-tagged PADI4 cell lysate treated with 10 mM $CaCl_2$ and 10 µM Ionomycin for 2 hours with ab202107 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab202107 at a dilution of 1/2000. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1/10000 dilution.

Lane 1: HEK-293 transfected with GFP-tagged PADI4 (WT) expression vector whole cell lysate treated with 10 mM $CaCl_2$ and 10 µM Ionomycin for 2 hours 10 µg (Input).

Lane 2: ab202107 IP in HEK-293 transfected with GFP-tagged PADI4 (WT) expression vector whole cell lysate treated with 10 mM $CaCl_2$ and 10 µM Ionomycin for 2 hours.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab202107 in HEK-293 transfected with GFP-tagged PADI4 (WT) expression vector whole cell lysate treated with 10 mM $CaCl_2$ and 10 µM Ionomycin for 2 hours.

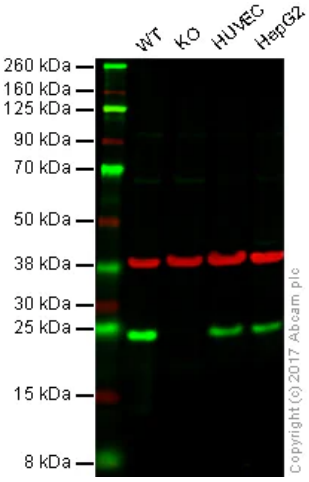
Exposure time: 10 seconds.

All lanes:
Immunoprecipitation - Anti-HP1 gamma/CBX3 (citrulline R108) antibody [EPR19802-202] (ab202107)

Predicted band size: 20 kDa

Observed band size: 21 kDa

Exposure time: 10s



Western blot - Anti-HP1 gamma/CBX3 (citrulline R108) antibody [EPR19802-202] - BSA and Azide free (ab251364)

HP1 gamma/CBX3 (citrulline R108) Western blot staining using rabbit Anti-HP1 gamma/CBX3 (citrulline R108) antibody

This data was developed using ab202107, the same antibody clone in a different buffer formulation.
Lane 1: Wild type HAP1 whole cell lysate (20 µg)

Lane 2: HP1 gamma/CBX3 knockout HAP1 whole cell lysate (20 µg)

Lane 3: HUVEC (umbilicalvein/vascular endothelium) whole cell lysate (20 µg)

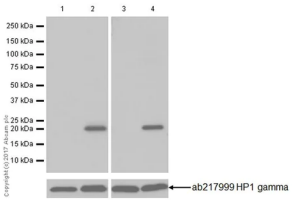
Lane 4: HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab202107 observed at 23 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab202107 was shown to specifically react with HP1 gamma/CBX3 when HP1 gamma/CBX3 knockout samples were used. Wild-type and HP1 gamma/CBX3 knockout samples were subjected to SDS-PAGE. Antibodies ab202107 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1000 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 1/10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-HP1 gamma/CBX3 (citrulline R108) antibody [EPR19802-202] (ab202107)

Predicted band size: 20 kDa



Western blot - Anti-HP1 gamma/CBX3 (citrulline R108) antibody [EPR19802-202] - BSA and Azide free (ab251364)

This data was developed using ab202107, the same antibody clone in a different buffer formulation.
Blocking and dilution buffer: 5% NFDM/TBST.

Exposure times: Lanes 1-2: 2 seconds; Lanes 3-4: 6 seconds.

All lanes:
Western blot - Anti-HP1 gamma/CBX3 (citrulline R108) antibody [EPR19802-202] (ab202107) at 1/1000 dilution

Lanes 1 and 3:
HEK-293 (human epithelial cell line from embryonic kidney) transfected with a control vector containing GFP tag treated with 10 mM calcium chloride and 10 µM Ionomycin for 2 hours, whole cell lysate at 20 µg

Lane 2:
HEK-293 (human epithelial cell line from embryonic kidney) transfected with GFP-tagged PADI4 (WT) expression vector, treated with 10 mM calcium chloride and 10 µM Ionomycin for 2 hours, whole cell lysate at 20 µg

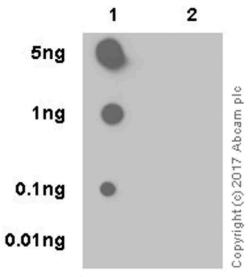
Lane 4:
HEK-293 (human epithelial cell line from embryonic kidney) transfected with GFP-tagged PADI2 (WT) expression vector treated with 10 mM calcium chloride and 10 µM Ionomycin for 2 hours, whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 20 kDa

Observed band size: 21 kDa



Dot Blot - Anti-HP1 gamma/CBX3 (citrulline R108) antibody [EPR19802-202] - BSA and Azide free (ab251364)

HP1 gamma/CBX3 (citrulline R108) Dot Blot staining using rabbit Anti-HP1 gamma/CBX3 (citrulline R108) antibody

This data was developed using ab202107, the same antibody clone in a different buffer formulation.
Dot blot analysis of HP1 gamma/CBX3 (citrulline R108) labeled with ab202107 at 1/1000 dilution.

Lane 1: HP1 gamma/CBX3 (citrulline R108) peptide;
Lane 2: HP1 gamma/CBX3 (non-citrulline) peptide;

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution was used as secondary antibody.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.