abcam

ab219406

Anti-Histone H3 (citrulline R8) antibody [EPR20358-13]

Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406) is a rabbit monoclonal antibody detecting Histone H3 in **Western Blot, Flow Cytometry (Intra), IP, ICC/IF, ELISA**. Suitable for **Human, Mouse, Rat**.

- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR20358-13
Purification technique	Affinity purification Protein A
Specificity	Treatment is required for detection of the target protein. See Protocol section for detailed information. Protocol .
Concentration	0.568 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

ELISA

Tested

Species	Synthetic peptide
Dilution info	-
Notes	-

Expected

Species	Human, Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000

Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Target data

See full target information H3C1 citrulline R8

Function	Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks

Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunocytochemistry/immunofluorescence (ICC/IF), ELISA in Human, Mouse, Rat samples.

What is the molecular weight of Histone H3?
Anti-Histone H3 (citrulline R8) [EPR20358-13] (ab219406) specifically detects a band for Histone H3 (UniProt: P68431) at a molecular weight of 15kDa.

Other related products
We have a range of other formats of antibody clone [EPR20358-13] also available for your convenience: ab219406, Carrier free - ab232939, Alexa Fluor® 647 - ab300306

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

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
- NIH3T3: $4\text{-}5x10^6$ cells

- C6: 3-4x10^6 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: 1/5000-1/10000 dilution at 4°C overnight or room temperature for 1 hour
Blocking: 5% BSA 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

ICC Conditions:
Primary: 1/100 dilution at 4°C overnight or room temperature for 2 hours
Fixation: 4% PFA at room temperature for 10-20 minutes
Permeabilization: 0.1 % Triton X-100 at room temperature for 2-5 minutes
Blocking: 2-10% serum from secondary host at room temperature for 1-2 hours

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

Additional resources:
ICC Protocol

Flow Cytometry Conditions:
Primary: 1/500 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

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

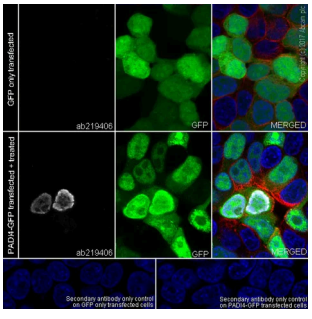
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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

Full details and terms and conditions can be found here:
Terms & Conditions.

8 product images



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

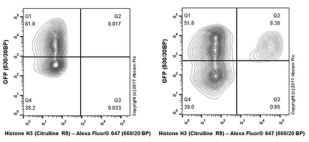
Histone H3 (citrulline R8) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Histone H3 (citrulline R8) antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T (Human epithelial cell line from embryonic kidney) cells transfected with GFP only or a GFP-tagged PADI4 expression construct, then treated with 10mM $CaCl_2$ for 2 hours, labeling Histone H3 (citrulline R8) with ab219406 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).
Confocal image showing positive staining on HEK-293T cells transfected with a GFP-tagged PADI4 expression construct, then treated with 10mM $CaCl_2$ for 2 hours.

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).

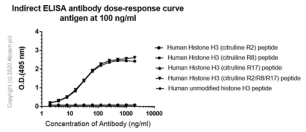
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

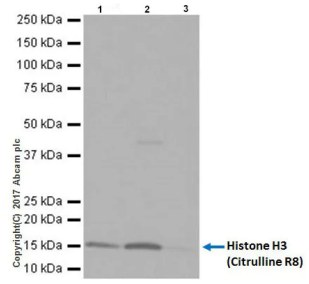
Histone H3 (citrulline R8) Flow Cytometry (Intracellular) staining using rabbit Anti-Histone H3 (citrulline R8) antibody

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HEK-293T (Human epithelial cell line from embryonic kidney) transfected with empty vector (left panel) or PADI4 (WT, right panel), then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours, labeling Histone H3 (citrulline R8)with ab219406 at 1/500 dilution, followed by Goat anti rabbit IgG (Alexa Fluor® 647) at 1/2000 dilution.
Positive signal is obtained from HEK-293T cells transfected with WT PADI4 treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours.



ELISA - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

ELISA analysis of Human Histone H3 (citrulline R8) recombinant protein at 100 ng/ml with ab219406. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

Histone H3 (citrulline R8) was immunoprecipitated from 0.35 mg of HEK-293T (Human epithelial cell line from embryonic kidney) transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate with ab219406 at 1/30 dilution.
Western blot was performed from the immunoprecipitate using ab219406 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

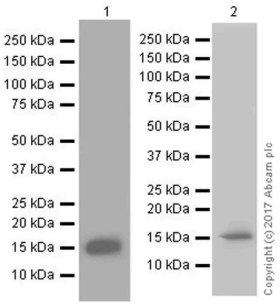
Lane 1: HEK-293T transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate 10 µg (Input).
Lane 2: ab219406 IP in HEK-293T transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate.
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab219406 in HEK-293T transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate.

Blocking and dilution buffer: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

Predicted band size: 15 kDa

Exposure time: 1s



Western blot - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

Histone H3 (citrulline R8) Western blot staining using rabbit Anti-Histone H3 (citrulline R8) antibody

Blocking/Dilution buffer: 5% BSA/TBST.
All lanes:
Western blot - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406) at 1/1000 dilution

Lane 1:
E12 rat embryo lysate at 10 µg

Lane 2:
E12 mouse embryo lysate at 10 µg

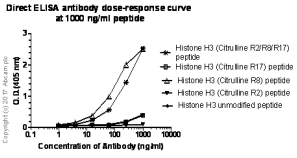
Secondary

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

Predicted band size: 15 kDa, 43 kDa

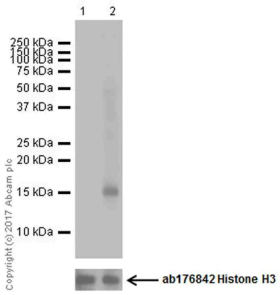
Observed band size: 15 kDa

Exposure time: 1s



ELISA - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

Direct ELISA using ab219406 at 0-1000 ng/ml, followed by Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) at 1/2500 dilution. Antigen concentration: 1000 ng/ml.



Western blot - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

Blocking/Dilution buffer: 5% BSA/TBST.
Histone H3R8 is citrullinated by PADI4 and $CaCl_2$ is used as a cofactor according to the literature (PMID: 16567635). Ionomycin is used to improve the modification by PADI4 according to the literature (PMID: 26360112).

All lanes:
Western blot - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406) at 1/10000 dilution

Lane 1:
Whole cell lysate from HEK-293T (Human epithelial cell line from embryonic kidney) transfected with empty vector with GFP tag (vector control), then treated with 10mM CaCl2 and 10µM Ionomyci at 10 µg

Lane 2:
Whole cell lysate from HEK-293T cells transfected with PADI4 (WT), then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours at 10 µg

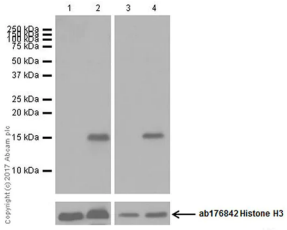
Secondary

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

Predicted band size: 15 kDa

Observed band size: 15 kDa

Exposure time: 1s



Western blot - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

Blocking/Dilution buffer: 5% BSA/TBST.
All lanes:
Western blot - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406) at 1/5000 dilution

Lane 1:
Whole cell lysate from NIH/3T3 (Mouse embryonic fibroblast cell line) transfected with empty vector with GFP tag (vector control), then treated with 10mM CaCl2 for 2 hours at 10 µg

Lane 2:
Whole cell lysate from NIH/3T3 transfected with PADI4 (WT) then treated with 10mM CaCl2 for 2 hours at 10 µg

Lane 3:
Whole cell lysate from C6 (Rat glial tumor cell line) transfected with empty vector with GFP tag (vector control) then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours at 10 µg

Lane 4:
C6 transfected with PADI4 (WT), then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours at 10 µg

Secondary

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

Predicted band size: 15 kDa

Observed band size: 15 kDa

Exposure time: 1s

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