abcam

ab232939

Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] - BSA and Azide free

Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] - BSA and Azide free (ab232939) is a rabbit recombinant monoclonal antibody for **Western Blot, Flow Cytometry (Intra), IP, ICC/IF, ELISA**. Suitable for **Human, Mouse, Rat**.

- BSA, sodium azide, and glycerol-free for easy conjugation
- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb

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	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	1.022 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

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

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

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

Not recommended

Species	Recombinant fragment - Human

Dilution info	-
Notes	-

ELISA

Tested

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

Expected

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

WB

Tested

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-

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	Recombinant fragment - Human
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	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

What is this antibody validated in?
Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] - BSA and Azide free (ab232939) 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] - BSA and Azide free (ab232939) 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

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
- NIH3T3: $4\text{-}5x10^6$ cells
- C6: $3\text{-}4x10^6$ 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% 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
Flow cytometry troubleshooting

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.

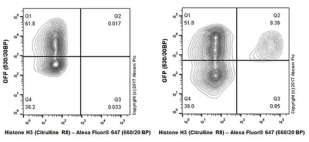
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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7 product images

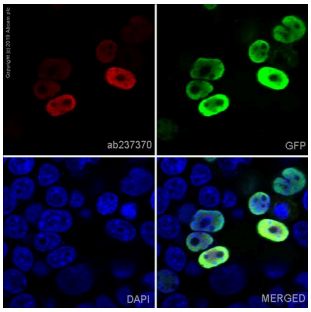


Flow Cytometry (Intracellular) - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] - BSA and Azide free (ab232939)

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 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 for 2 hours.

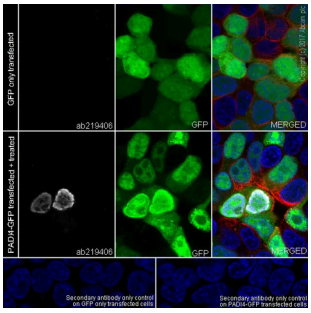
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219406).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] - BSA and Azide free (ab232939)

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

Clone EPR20358-13 (ab232939) has been successfully conjugated by Abcam. This image was generated using Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (Alexa Fluor® 647). Please refer to ab237370 for protocol details.
Immunofluorescent analysis of 4% PFA-fixed, 0.1% Triton X-100 permeabilized HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) cells labeling Histone H3 with ab237370 at 1/100 dilution (red). Confocal image showing nuclear staining in HEK-293T cells transfected with a GFP-tagged PADI4 expression construct (green), then treated with 10mM $CaCl_2$ for 2 hours and 10µM Ionomycin for 2 hours. Nuclear counterstain is DAPI (blue).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] - BSA and Azide free (ab232939)

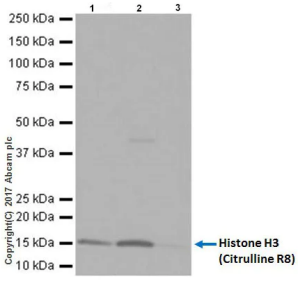
Histone H3 (citrulline R8) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Histone H3 (citrulline R8) antibody

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 10 mM $CaCl_2$ 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219406).



Immunoprecipitation - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] - BSA and Azide free (ab232939)

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 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 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 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 for 2 hours, whole cell lysate.

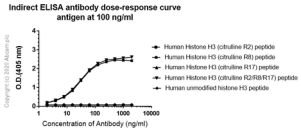
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219406).
All lanes:
Immunoprecipitation - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

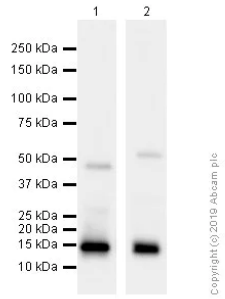
Predicted band size: 15 kDa

Exposure time: 1s



ELISA - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] - BSA and Azide free (ab232939)

This data was developed using ab219406, the same antibody clone in a different buffer formulation.
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.



Western blot - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] - BSA and Azide free (ab232939)

All lanes:
Western blot - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] - BSA and Azide free (ab232939) at 1/1000 dilution

Lane 1:
E12 mouse embryo lysates at 15 µg

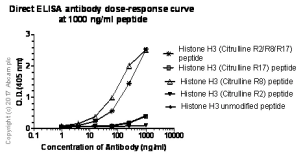
Lane 2:
E12 rat embryo lysates at 15 µg

Secondary

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

Predicted band size: 15 kDa

Exposure time: 6s



ELISA - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] - BSA and Azide free (ab232939)

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. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219406).

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