abcam

ab283863

Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] - BSA and Azide free

Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] - BSA and Azide free (ab283863) is a rabbit recombinant recombinant multiclonal antibody for **Western Blot, Flow Cytometry (Intra), IP, ICC/IF, Dot Blot, PepArr**. Suitable for **Human, Mouse, Rat**.

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

Recombinant | RabMAb | Advanced Validation

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Multiclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	RM1001
Purification technique	Affinity purification Protein A
Specificity	Treatment is required for detection of the target protein. See Protocol section for detailed information. Protocol .
Concentration	0.998 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Dot

Tested

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Expected

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

IHC-P

Not recommended

Species	Rat, Mouse, Human, Synthetic peptide - Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	The up-regulation of PADI4 expression can induce the citrullination modification of Histone H3. Therefore, we suggest choosing cells overexpressing the PADI4 protein as the positive control.

Species	Mouse
Dilution info	-
Notes	The up-regulation of PADI4 expression can induce the citrullination modification of Histone H3. Therefore, we suggest choosing cells overexpressing the PADI4 protein as the positive control.

Species	Rat

Dilution info	-
Notes	The up-regulation of PADI4 expression can induce the citrullination modification of Histone H3. Therefore, we suggest choosing cells overexpressing the PADI4 protein as the positive control.

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

ChIP

Not recommended

Species	Human, Rat, Mouse, Synthetic peptide - Human
Dilution info	-
Notes	-

PepArr

Tested

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Expected

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

IP

Tested

Species	Human

Dilution info	-
Notes	-

Expected

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

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Expected

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

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

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

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Target data

See full target information H3C1 citrulline R2 + R8 + R17

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 R2 + R8 + R17) antibody [RM1001] - BSA and Azide free (ab283863) is a rabbit recombinant recombinant multiclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunocytochemistry/immunofluorescence (ICC/IF), Dot Blot, Peptide Array in Human, Mouse, Rat samples.

What is the molecular weight of Histone H3?
Anti-Histone H3 (citrulline R2 + R8 + R17) [RM1001] - BSA and Azide free (ab283863) 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 [RM1001] also available for your convenience: ab281584, Carrier free - ab283863

What are recombinant multiclonals?
Recombinant multiclonals are a mixture of recombinant antibodies co-expressed from a library of heavy and light chains. They offer several advantages including:

- The sensitivity of polyclonal antibodies by recognising multiple epitopes
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

View our range of recombinant multiclonal antibodies.

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.

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% 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

ICC Conditions:
Primary: 1/1000 dilution at 4°C overnight or room temperature for 2 hours
Fixation: 4% PFA at room temperature for 10-20 minutes
Permeabilization: 100% Methanol 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: 90% Methanol

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

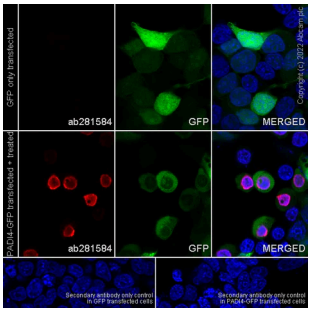
We do not recommend this combination. It is not covered by our product promise.

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.

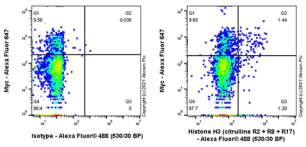
7 product images



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] - BSA and Azide free (ab283863)

Histone H3 (citrulline R2 + R8 + R17) Immunocytochemistry/ Immunofluorescence staining of 293T cells transfected with a human PADI4 expression vector containing a GFP tag using rabbit Anti-Histone H3 (citrulline R2 + R8 + R17) antibody

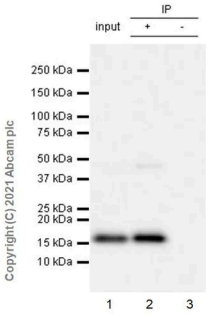
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 100% methanol permeabilized 293T (human embryonic kidney epithelial cell) cells labelling Histone H3 (citrulline R2 + R8 + R17) with primary antibody anti-Histone H3 (citrulline R2 + R8 + R17) (ab281584) at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) (ab150080) secondary antibody at 1/1000 dilution. Confocal image showing nuclear staining on 293T cells transfected with a human PADI4 expression vector containing a GFP tag, then treated with 10 mM CaCl2 and 10 µM ionomycin for 2 hours. The nuclear counter stain is DAPI (blue). This data was developed using ab281584, the same antibody clone in a different buffer formulation.



Flow Cytometry (Intracellular) - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] - BSA and Azide free (ab283863)

Histone H3 (citrulline R2 + R8 + R17) Flow Cytometry (Intracellular) staining using rabbit Anti-Histone H3 (citrulline R2 + R8 + R17) antibody

Intracellular Flow Cytometry analysis of 293T (human embryonic kidney epithelial cell) transfected with myc-tagged PADI4 construct, then treated with 10mM CaCl2 and 10uM Ionomycin for 4h labeling Histone H3 (citrulline R2 + R8 + R17) with ab281584 at 1/500 dilution. Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as a secondary antibody. Isotype control : Rabbit monoclonal IgG (ab172730)/left. This data was developed using ab281584, the same antibody clone in a different buffer formulation.



Immunoprecipitation - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] - BSA and Azide free (ab283863)

This data was developed using ab281584, the same antibody clone in a different buffer formulation.Histone H3 (citrulline R2 + R8 + R17) was immunoprecipitated from 0.35 mg HEK-293T (human embryonic kidney) transfected with PADI4 expression vector containing a GFP-tag treated with 10 mM calcium chloride and 10 µM lonomycin for 4 hours, whole cell lysate 10 µg with 281584 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using 281584 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.
Lane 1: HEK-293T (human embryonic kidney) transfected with PADI4 expression vector containing a GFP-tag treated with 10 mM calcium chloride and 10 µM lonomycin for 4 hours, whole cell lysate 10 µg.

Lane 2: ab281584 IP in HEK-293T transfected with PADI4 expression vector containing a GFP-tag treated with 10 mM calcium chloride and 10 µM lonomycin for 4 hours whole cell lysate

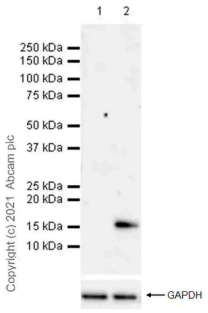
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab281584 in HEK-293T transfected with PADI4 expression vector containing a GFP-tag treated with 10 mM calcium chloride and 10 µM lonomycin for 4 hours whole cell lysate

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

Exposure time: 10 seconds

All lanes:
Immunoprecipitation - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] - BSA and Azide free (ab283863)

Predicted band size: 15 kDa



Western blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] - BSA and Azide free (ab283863)

Histone H3 (citrulline R2 + R8 + R17) Western blot staining using rabbit Anti-Histone H3 (citrulline R2 + R8 + R17) antibody

This data was developed using ab281584, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 180 seconds

All lanes:
Western blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] - BSA and Azide free (ab283863) at 1/1000 dilution

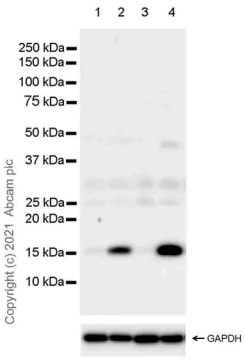
Lane 1:
Untreated C6 (rat glial tumor glial cell) transfected with PADI4 expression vector containing a GFP-tag, whole cell lysate at 20 µg

Lane 2:
C6 transfected with PADI4 expression vector containing a GFP-tag 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: 15 kDa



Western blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] - BSA and Azide free (ab283863)

Histone H3 (citrulline R2 + R8 + R17) Western blot staining using rabbit Anti-Histone H3 (citrulline R2 + R8 + R17) antibody

This data was developed using ab281584, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 48 seconds

All lanes:
Western blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] - BSA and Azide free (ab283863) at 1/1000 dilution

Lane 1:
Untreated HEK-293T (human embryonic kidney) transfected with PADI4 expression vector containing a myc-His-tag®, whole cell lysate at 20 µg

Lane 2:
HEK-293T transfected with PADI4 expression vector containing a myc-His-tag® treated with 10 mM calcium chloride and 10 µM Ionomycin for 4 hours, whole cell lysate at 20 µg

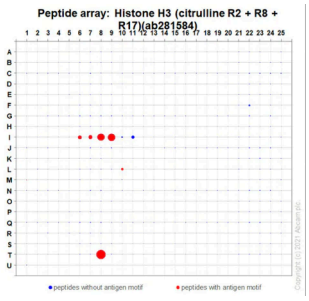
Lane 3:
Untreated NIH/3T3 mouse embryonic fibroblast transfected with PADI4 expression vector containing a myc-His-tag®, whole cell lysate at 20 µg

Lane 4:
NIH/3T3 transfected with PADI4 expression vector containing a myc-His-tag® treated with 10 mM calcium chloride and 10 µM Ionomycin for 4 hours, whole cell lysate at 20 µg

Secondary

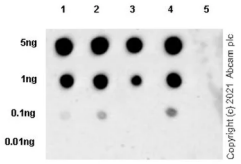
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 15 kDa



Peptide Array - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] - BSA and Azide free (ab283863)

This data was developed using ab281584, the same antibody clone in a different buffer formulation. Peptide array analysis of ab281584 at (0.1ug/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Fluo 647nm conjugated at 1:50,000 dilution. All batches of ab281584 are tested in Peptide Array against 501 different modified and unmodified histone peptides; each peptide is printed on the array at six concentrations (each in triplicate). Circle area represents affinity between the antibody and a peptide: all antigen-containing peptides are displayed as red circles, all other peptides as blue circles. The affinity is calculated as area under curve when antibody binding values are plotted against the corresponding peptide concentration. Each circle area is normalized to the peptide with the strongest affinity. The complete dataset, including full list of all peptides and information on the position of each peptide in the diagram, can be downloaded from the 'documents' section.



Dot Blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] - BSA and Azide free (ab283863)

This data was developed using ab281584, the same antibody clone in a different buffer formulation.Dot blot analysis of Histone H3 (citrulline R2 + R8 + R17) using 281584 at 1/1000 (0.478 ug/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/100,000 dilution.
Lane 1: Histone H3 (citrulline R2) peptide (aa 1-21)

Lane 2: Histone H3 (citrulline R8) peptide (aa 1-21)

Lane 3: Histone H3 (citrulline R17) peptide (aa 1-21)

Lane 4: Histone H3 (citrulline R2/R8/R17) peptide (aa 1-21)

Lane 5: Unmodified histone H3 peptide (aa 1-21)

Exposure time: 3 minutes

Blocking and diluting buffer and concentration: 5% NFDM/TBST

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