abcam

ab281584

Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001]

Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584) is a rabbit recombinant multiclonal antibody detecting Histone H3 in **Western Blot, Flow Cytometry (Intra), IP, ICC/IF, Dot Blot, PepArr**. Suitable for **Human, Mouse, Rat**.

- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | Advanced Validation | 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	Multiclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
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	0.529 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

PepArr

Tested

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Expected

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

Dot

Tested

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Expected

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

WB

Tested

Species	Mouse
Dilution info	1/1000
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	1/1000
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	Human
Dilution info	1/1000
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	-

IHC-P

Not recommended

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

ChIP

Not recommended

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

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 - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
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	1/2000
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 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

Product Specifications
Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584) is a recombinant rabbit multiclonal antibody which is comprised of multiple monoclonal antibodies targeting different epitopes and recapitulating the performance of a polyclonal. Each component clone was developed by Abcam using patented rabbit monoclonal antibody technology.
Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584) is validated for use in Dot blot, Flow Cyt (Intra), ICC/IF, IP, PepArr and WB in human, mouse and rat samples.
Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584) specifically detects Histone H3 (citrulline R2 + R8 + R17) (UniProt ID: P68431; Molecular weight: 15kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Quality and Validation
Abcams high quality manufacturing and validation processes ensure anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

Related Products
Conjugation-ready, carrier free format available for antibody clone RM1001 - ab283863.

Target Information
This antibody is a key tool in cancer research, particularly in understanding histone citrullination's role in tumorigenesis, and is widely used in studies of gene expression, DNA repair, and autoimmune diseases like rheumatoid arthritis and lupus, involving protein arginine deiminases (PADs) and NETosis. Histone hypercitrullination is reported to play a role the progression of various cancers, including multiple myeloma, acute myeloid leukemia , lung cancer, and hepatocellular carcinoma.

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.

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

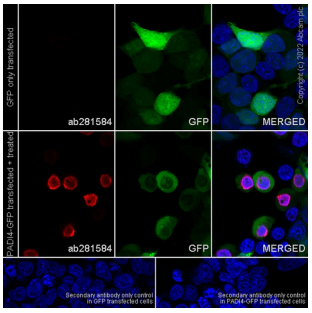
We do not recommend this combination. It is not covered by our product promise.

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:
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7 product images

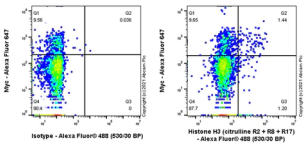


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

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

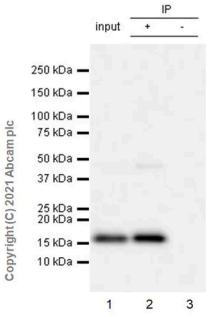
primary antibody anti-Histone H3 (citrulline R2 + R8 + R17) (ab281584) at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) (ab150080) secondary antibody at 1/1000 dilution. Confocal image showing nuclear staining on 293T cells transfected with a human PADI4 expression vector containing a GFP tag, then treated with 10 mM CaCl2 and 10 µM ionomycin for 2 hours. The nuclear counter stain is DAPI (blue).



Flow Cytometry (Intracellular) - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

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.



Immunoprecipitation - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

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

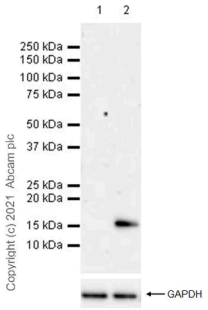
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab281584 in HEK-293T transfected with PADI4 expression vector containing a GFP-tag treated with 10 mM calcium chloride and 10 µM lonomycin for 4 hours whole cell lysate

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] (ab281584)

Predicted band size: 15 kDa



Western blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

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

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] (ab281584) at 1/1000 dilution

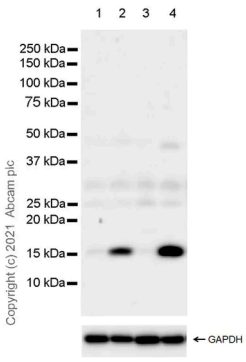
Lane 1:
Untreated C6 (rat glial tumor glial cell) transfected with PADI4 expression vector containing a GFP-tag, whole cell lysate at 20 µg

Lane 2:
C6 transfected with PADI4 expression vector containing a GFP-tag treated with 10 mM calcium chloride and 10 µM lonomycin 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] (ab281584)

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

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] (ab281584) 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 lonomycin 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

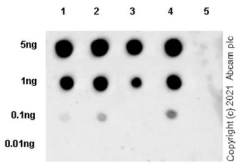
Lane 4:
NIH/3T3 transfected with PADI4 expression vector containing a myc-His-tag® treated with 10 mM calcium chloride and 10 µM lonomycin for 4 hours, whole cell lysate at 20 µg

Secondary

All lanes:

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

Predicted band size: 15 kDa



Dot Blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

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)

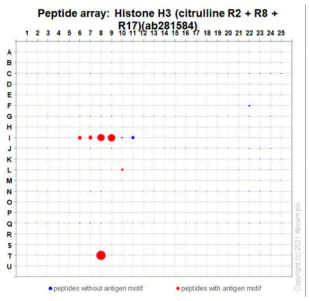
Lane 3: Histone H3 (citrulline R17) peptide (aa 1-21)

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



Peptide Array - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

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.

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