abcam

ab32388

Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade

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, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	E191
Purification technique	Affinity purification Protein A
Specificity	This antibody detects Histone H3 and Histone H3.3 when phosphorylated on Serine 28. It does not detect H3.3 when phosphorylated on Serine 31.
Concentration	1.265 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

PepArr

Tested

Species	Synthetic peptide

Dilution info	0.02 µg/mL
Notes	-

Expected

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

Predicted

Species	Rat, Guinea pig
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Expected

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

Predicted

Species	Rat, Guinea pig
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/60
Notes	-

Expected

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

Predicted

Species	Rat, Guinea pig
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

ChIP

Tested

Species	Human
Dilution info	5 µg for 25 µg chromatin
Notes	-

Expected

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

Predicted

Species	Rat, Guinea pig
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Dot

Tested

Species	Synthetic peptide
Dilution info	-
Notes	-

Expected

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

Predicted

Species	Rat, Guinea pig
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/2000
Notes	-

Species	Human
Dilution info	1/2000
Notes	-

Predicted

Species	Rat, Guinea pig
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/250

Notes	-

Expected

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

Predicted

Species	Rat, Guinea pig
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100 - 1/1200
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

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

Predicted

Species	Rat, Guinea pig
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Target data

See full target information H3-4 phospho S28

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

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.

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

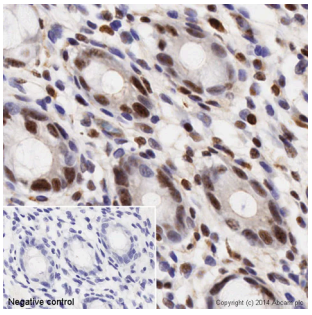
We do not recommend this combination. It is not covered by our product promise.

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.

11 product images

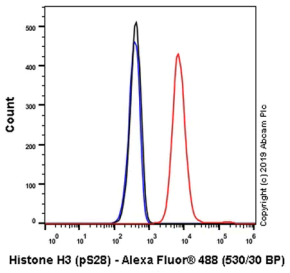


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Histone H3 (phospho S28) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Histone H3 (phospho S28) antibody

IHC image of ab32388 staining Histone H3 in Human normal colon formalin fixed paraffin embedded tissue* sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab32388, 0.1ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the negative control (shown on the inset).
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

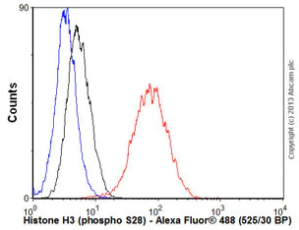
*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Flow Cytometry (Intracellular) - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Histone H3 (phospho S28) Flow Cytometry (Intracellular) staining using rabbit Anti-Histone H3 (phospho S28) antibody

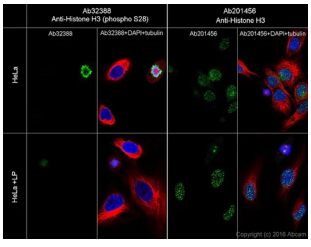
Overlay histogram showing HeLa cells stained with ab32388 (red line). The cells were fixed with 4% paraformaldehyde and then permeabilized with 90% methanol. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32388, 1/1200 dilution, 1.02 µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (ab172730) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Flow Cytometry (Intracellular) - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Histone H3 (phospho S28) Flow Cytometry (Intracellular) staining using rabbit Anti-Histone H3 (phospho S28) antibody

Overlay histogram showing HeLa cells stained with ab32388 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32388, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

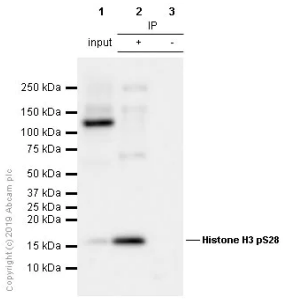


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Histone H3 (phospho S28) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Histone H3 (phospho S28) antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% tritonX-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, LP starved and non-starved, labeling anti-Histone H3 (phospho S28) with ab32388 at 1/100 dilution followed by Goat anti-Rabbit secondary IgG AlexaFluor®488 (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on M phase of HeLa cells, then the signal decreased after LP treatment.

For the pan antibody, there was no great difference after LP treatment. The data showed mostly nuclear staining



Immunoprecipitation - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Immunoprecipitation dilution was 1/60.
Western blot was performed on the immunoprecipitate using ab32388 at 1/500 dilution (2.444 µg/ml). VeriBlot for IP secondary antibody (HRP) (ab131366) at 1:1000 dilution.

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

All lanes:
Immunoprecipitation - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

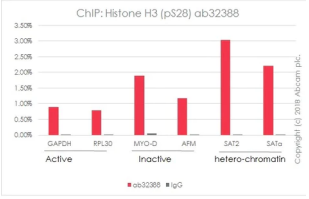
Lane 1:
HeLa (human cervix adenocarcinoma epithelial cell) treated with 0.5µM Nocodazole for 24h whole cell lysate at 10 µg

Lane 2:
ab32388 IP in Nocodazole treated HeLa whole cell lysate

Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab32388 in nocodazole treated HeLa whole cell lysate

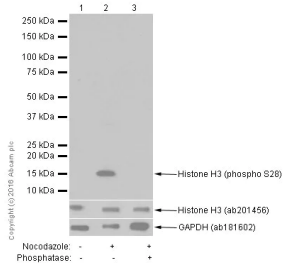
Predicted band size: 15 kDa

Observed band size: 17 kDa



ChIP - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min.
The ChIP was performed with 25 µg of chromatin, 5 µg of ab32388 (red), and 20 µl of Protein A/G sepharose beads. Rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).
Primers and probes are located in the first kb of the transcribed region.



Western blot - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Histone H3 (phospho S28) Western blot staining using rabbit Anti-Histone H3 (phospho S28) antibody

Blocking/dilution buffer: 2% BSA/TBST

All lanes:
Western blot - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388) at 1/1000 dilution

Lane 1:
HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 15 µg

Lane 2:
Whole cell lysate from HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, treated with 100ng/ml nocodazole for 18 hours at 15 µg

Lane 3:
Whole cell lysate from HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, treated with 100ng/ml nocodazole for 18 hours. Membrane incubated with phosphatase at 15 µg

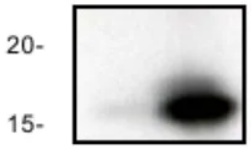
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 15 kDa

Observed band size: 17 kDa

Exposure time: 15s



Western blot - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

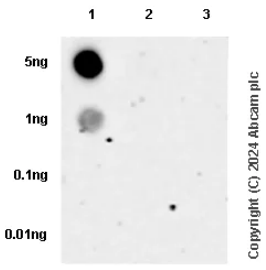
All lanes:
Western blot - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388) at 1/2000 dilution

Lane 1:
NIH 3T3 cell lysate -untreated

Lane 2:
NIH 3T3 cell lysate -treated with FBS + CalA.

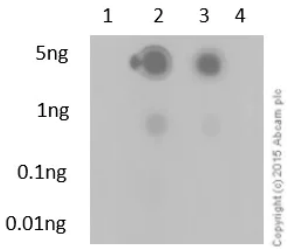
Predicted band size: 15 kDa

Observed band size: 17 kDa



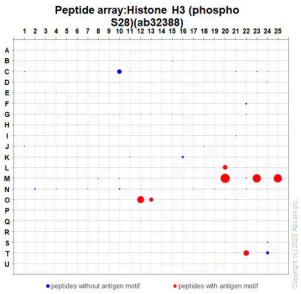
Dot Blot - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Dot blot analysis of Histone H3 (phospho S28) labeled with ab32388 at 1/1000 dilution. Lane 1: Histone H3 (phospho S28) peptide a Lane 2: Histone H3 (phospho S28) peptide c1 Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) was used as secondary antobody at 1/100000 dilution. Blocking/Dilution buffer: 5% NFDM/TBST. Exposure time: 180 minutes.



Dot Blot - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Dot blot performed using ab32388 at a dilution of 1/100. Lane 1 - Unmodified H3 peptide. Lane 2 - H3S28ph peptide. Lane 3 - H3.3S28ph peptide. Lane 4 - H3.3S31ph peptide. A HRP conjugated goat anti-rabbit (H+L) was used as the secondary antibody at a dilution of 1/2500. The exposure time was 3 minutes and the dilution and blocking buffer used were 5% NFDM/TBST.



Peptide Array - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

ab32388 (0.02µg/mL) was 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). A Goat Anti-Rabbit IgG, (H+L), Fluo 647nm conjugated at 1/50000 was used as the secondary antibody. Protein Array Washing Buffer, 2*10 mins and 2*5 mins. Blocking buffer was 5% BSA in TBST and Innoscan 710 scanner was used. 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 the area under the 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, is available under the Product Protocol section.

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