abcam

ab215532

Anti-Histone H3 (phospho S28) antibody [E191] - BSA and Azide free

Recombinant | RabMAb | Advanced Validation

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

Reactivity data

PepArr

Tested

Species	Synthetic peptide
Dilution info	-
Notes	-

Expected

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

Predicted

Species	Mouse, 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.

Predicted

Species	Mouse, Rat, Guinea pig
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-

Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat, Guinea pig
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

ChIP

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

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

Predicted

Species	Mouse, Rat, Guinea pig
Dilution info	-
Notes	-

WB

Expected

Species	Mouse, Human
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	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat, Guinea pig
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-

Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Predicted

Species	Mouse, 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	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab215532 is the carrier-free version of ab32388.

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.

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.

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

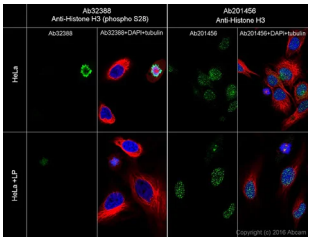
We do not recommend this combination. It is not covered by our product promise.

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.

9 product images



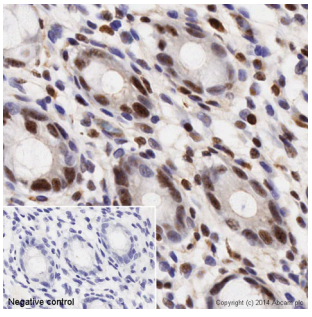
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S28) antibody [E191] - BSA and Azide free (ab215532)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32388).



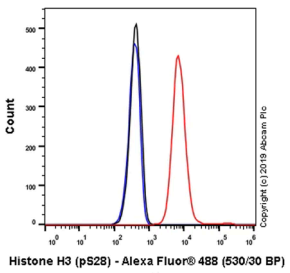
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (phospho S28) antibody [E191] - BSA and Azide free (ab215532)

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

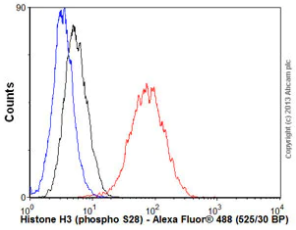
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32388).



Flow Cytometry (Intracellular) - Anti-Histone H3 (phospho S28) antibody [E191] - BSA and Azide free (ab215532)

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

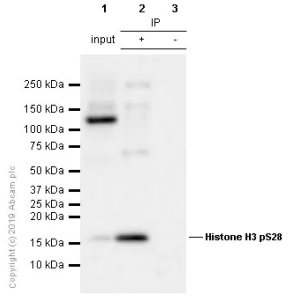
Overlay histogram showing HeLa cells stained with ab32388 (red line). The cells were fixed with 4% paraformaldehyde and then permeabilized with 90% methanol. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the 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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32388).



Flow Cytometry (Intracellular) - Anti-Histone H3 (phospho S28) antibody [E191] - BSA and Azide free (ab215532)

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. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32388).



Immunoprecipitation - Anti-Histone H3 (phospho S28) antibody [E191] - BSA and Azide free (ab215532)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32388).

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

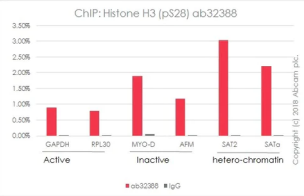
Lane 1:
HeLa (human cervix adenocarcinoma epithelial cell) treated with 0.5µM Nocodazole for 24h whole cell lysate at 10 µg

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

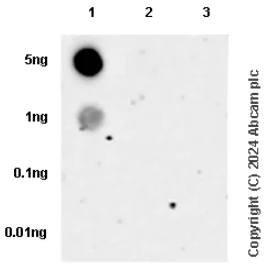
Predicted band size: 15 kDa

Observed band size: 17 kDa



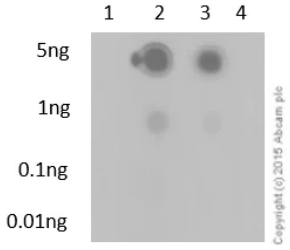
ChIP - Anti-Histone H3 (phospho S28) antibody [E191] - BSA and Azide free (ab215532)

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. No antibody 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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32388).



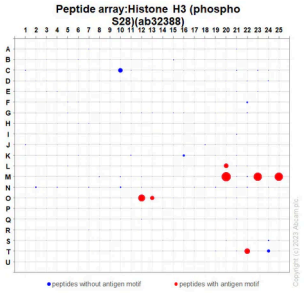
Dot Blot - Anti-Histone H3 (phospho S28) antibody [E191] - BSA and Azide free (ab215532)

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. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32388).



Dot Blot - Anti-Histone H3 (phospho S28) antibody [E191] - BSA and Azide free (ab215532)

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.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32388).



Peptide Array - Anti-Histone H3 (phospho S28) antibody [E191] - BSA and Azide free (ab215532)

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