abcam

ab176842

Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade

Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) is a rabbit monoclonal antibody for Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF, ChIP, PepArr. Suitable for Drosophila, Human, Mouse, Rat, S.cerevisiae, S.pombe. - Biophysical QC for unrivalled batch-batch consistency - Over 70 publications

Lab Essentials | Recombinant | RabMAb | Advanced Validation | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR16987
Purification technique	Affinity purification Protein A
Concentration	0.496 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ChIP

Tested

Species	Human

Dilution info	2 µg for 25 µg chromatin
Notes	-

Expected

Species	Mouse, Rat, Schizosaccharomyces pombe, Saccharomyces cerevisiae, Drosophila melanogaster
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Sheep, Dog, Common marmoset
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1 µg/mL
Notes	-

Species	Rat
Dilution info	1 µg/mL
Notes	-

Species	Schizosaccharomyces pombe
Dilution info	1 µg/mL
Notes	-

Species	Saccharomyces cerevisiae
Dilution info	1 µg/mL
Notes	-

Species	Drosophila melanogaster

Dilution info	1 µg/mL
Notes	-

Species	Human
Dilution info	1 µg/mL
Notes	-

Predicted

Species	Sheep, Dog, Common marmoset
Dilution info	-
Notes	-

PepArr

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat, Schizosaccharomyces pombe, Saccharomyces cerevisiae, Drosophila melanogaster
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Sheep, Dog, Common marmoset
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/2000 - 1/5000
Notes	-

Expected

Species	Mouse, Rat, Schizosaccharomyces pombe, Saccharomyces cerevisiae, Drosophila melanogaster
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Sheep, Dog, Common marmoset
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat, Schizosaccharomyces pombe, Saccharomyces cerevisiae, Drosophila melanogaster
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Sheep, Dog, Common marmoset
Dilution info	-

Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	1/5000 - 1/10000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/5000 - 1/10000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Human
Dilution info	1/5000 - 1/10000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Expected

Species	Schizosaccharomyces pombe, Saccharomyces cerevisiae, Drosophila melanogaster
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Sheep, Dog, Common marmoset
Dilution info	-
Notes	-

Target data

See full target information H3C1

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

What is this antibody validated in?
Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF), ChIP, Peptide Array in Drosophila melanogaster, Human, Mouse, Rat, Saccharomyces cerevisiae, Schizosaccharomyces pombe samples.

What is the molecular weight of Histone H3?
Anti-Histone H3 [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) specifically detects a band for Histone H3 (UniProt: P68431) at a molecular weight of 15kDa.

Trusted by the scientific community
Anti-Histone H3 [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) was first used in a scientific publication in 2014 and has been cited over 70 times in peer-reviewed journals.

Reviewed by scientists
Anti-Histone H3 [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) has over 10 independent reviews from customers.

Specificity confirmed
The specificity of Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) has been confirmed by Western blot testing in H3C1 Knockout HAP1 cells.

Other related products

We have a range of other formats of antibody clone [EPR16987] also available for your convenience: ab176842, Alexa Fluor® 647 - ab207543, HRP - ab209023, Carrier free - ab238971, Alexa Fluor® 488 - ab252005, PE - ab314246, Alexa Fluor® 750 - ab321187

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.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size 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

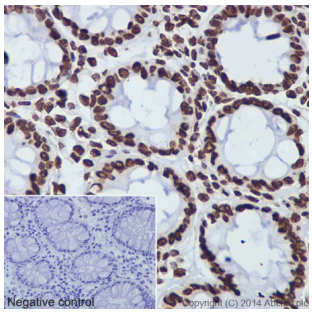
We do not recommend this combination. It is not covered by our product promise.

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.

40 product images

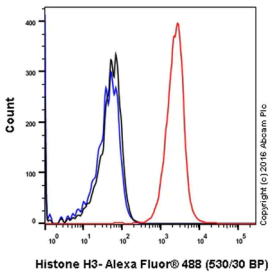


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

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

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Histone H3 with ab176842 at 1/500 dilution, followed by Goat Anti-Rabbit HRP (IgG; H&L) secondary antibody (ab97051) at 1/500 dilution. Nuclear staining on glandular epithelium of Human colon tissue is observed. Counter stained with Hematoxylin.
Negative control: Used PBS instead of primary antibody, secondary antibody Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

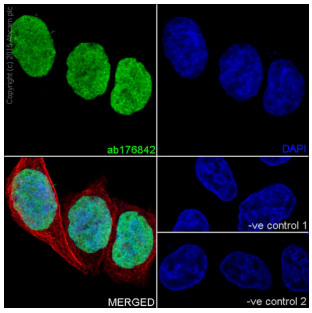
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

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

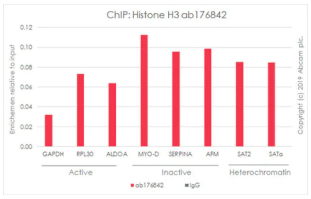
Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling Histone H3 with purified ab176842 at 1/40 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

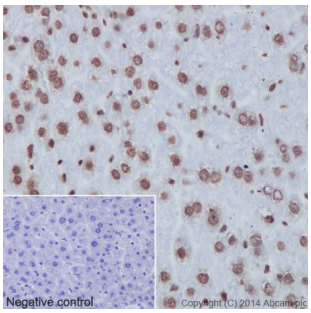
Histone H3 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Histone H3 antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Histone H3 with ab176842 at 1/2000 dilution, followed by Goat anti-rabbit Alexa Fluor® 488 (IgG) (ab150077) secondary antibody at 1/400 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/500 dilution and ab150120 (goat anti-mouse AlexaFluor®594 Goat secondary antibody) at 1/500 dilution (red).
The negative controls are as follows;
-ve control 1: ab176842 at 1/2000 dilution followed by ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: ab7291 (anti-Tubulin mouse mAb) at 1/500 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/400 dilution.



ChIP - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab176842 (red), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are located in the first kb of the transcribed region.

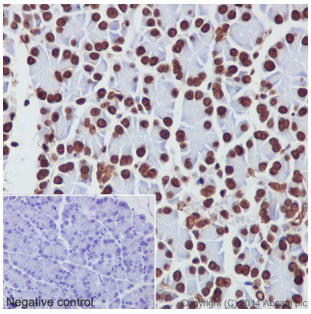


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

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

Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling Histone H3 with ab176842 at 1/500 dilution, followed by Goat Anti-Rabbit HRP (IgG H&L) secondary antibody (ab97051) at 1/500 dilution. Nuclear staining on mouse liver tissue is observed. Counter stained with Hematoxylin.
Negative control: Used PBS instead of primary antibody, secondary antibody Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

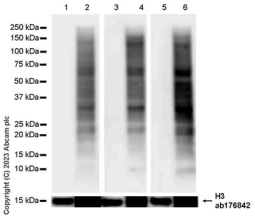


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

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

Immunohistochemical analysis of paraffin-embedded Rat pancreas tissue labeling Histone H3 with ab176842 at 1/500 dilution, followed by Goat Anti-Rabbit HRP (IgG H&L) secondary antibody (ab97051) at 1/500 dilution. Nuclear staining on rat pancreas tissue is observed. Counter stained with Hematoxylin.
Negative control: Used PBS instead of primary antibody, secondary antibody Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

Puromycin is a naturally occurring aminonucleoside antibiotic that inhibits protein synthesis by ribosome-catalyzed incorporation into the C-terminus of elongating nascent chains, blocking further extension and resulting in premature termination of translation.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

Exposure time: Lane 1-2: 1 second, Lane 3-4: 6 seconds, Lane 5-6: 3 seconds.
All lanes:
Western blot - Anti-Puromycin antibody [EPR27218-173] (ab315887) at 1/1000 dilution

Lane 1:
Untreated HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
HeLa treated with 1uM Puromycin for 30min whole cell lysate at 20 µg

Lane 3:
Untreated NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

Lane 4:
NIH/3T3 treated with 1uM Puromycin for 30min whole cell lysate at 20 µg

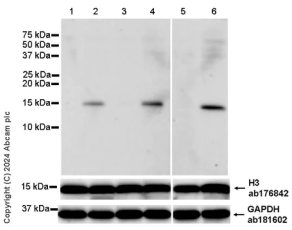
Lane 5:
Untreated C6 (rat glial tumor glial cell) whole cell lysate at 20 µg

Lane 6:
C6 treated with 1uM Puromycin for 30min whole cell lysate at 20 µg

Secondary

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

Observed band size: 10-250 kDa, 15 kDa



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

The expression of Histone H3 (propionyl K23) is upregulated in response to sodium propionate treatment (PMID: 32010779).

The identity of the bands above 37 kDa are unknown.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

All lanes:
Western blot - Anti-Histone H3 (propionyl K23) antibody [EPR28672-86] (ab317819) at 1/1000 dilution

Lane 1:
Untreated HepG2 (human hepatocellar carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
HepG2 treated with 10mM sodium propionate for 4 hours whole cell lysate at 20 µg

Lane 3:
Untreated HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
Hela treated with 10mM sodium propionate for 4 hours whole cell lysate at 20 µg

Lane 5:
Untreated MEF (mouse embryo fibroblast) whole cell lysate at 20 µg

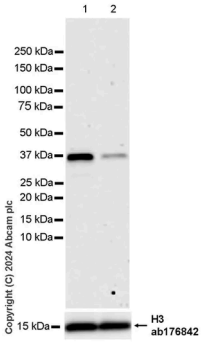
Lane 6:
MEF treated with 10mM sodium propionate for 24 hours whole cell lysate at 20 µg

Secondary

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

Observed band size: 15 kDa, 36 kDa

Exposure time: 180s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

All lanes:
Western blot - Anti-AIMP2/p38 antibody [EPR29127-48] (ab316937) at 1/1000 dilution

Lane 1:
HeLa (human cervical adenocarcinoma epithelial cell) transfected with scrambled siRNA control whole cell lysate at 20 µg

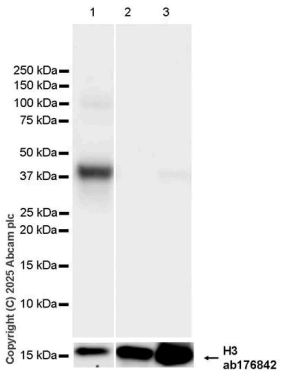
Lane 2:
HeLa transfected with siRNA specifically targeting AIMP2 whole cell lysate at 20 µg

Secondary

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

Observed band size: 35 kDa, 15 kDa

Exposure time: 180s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

Low expression: pancreas, colon.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

All lanes:
Western blot - Anti-Aldolase C antibody [EPR26896-61] - C-terminal (ab323506) at 1/1000 dilution

Lane 1:
Human cerebellum tissue lysate at 20 µg

Lane 2:
Human pancreas tissue lysate at 20 µg

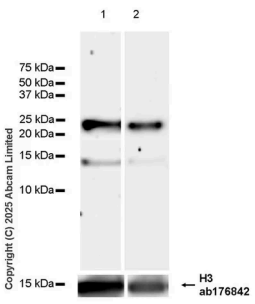
Lane 3:
Human colon tissue lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Observed band size: 39 kDa, 15 kDa

Exposure time: 1s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

The identity of the lower MW band at approximately 15 kDa is unknown.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) 1:100000 (15Kda).

Exposure time: Lane 1: 180 seconds, lane 2: 26 seconds

All lanes:
Western blot - Anti-Histone H2A (ubiquityl K119) antibody [EPR28331-576] (ab325796) at 1/1000 dilution

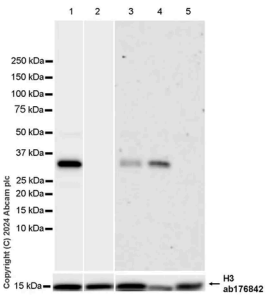
Lane 1:
HeLa (human cervical adenocarcinoma epithelial cell) histone fraction at 20 µg

Lane 2:
NIH/3T3 (mouse embryonic fibroblast) histone fraction at 20 µg

Secondary

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

Observed band size: 22 kDa, 15 kDa



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

Negative control: Jurkat

Low expression: liver

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

Exposure time: Lane1-2: 15seconds, lane3-5: 180 seconds

All lanes:
Western blot - Anti-MNDA antibody [EPR28267-44] (ab316967) at 1/1000 dilution

Lane 1:
U-937 (human histiocytic lymphoma monocyte) whole cell lysate at 20 µg

Lane 2:
Jurkat (human t cell leukemia t lymphocyte from peripheral blood) whole cell lysate at 20 µg

Lane 3:
Human spleen tissue lysate at 20 µg

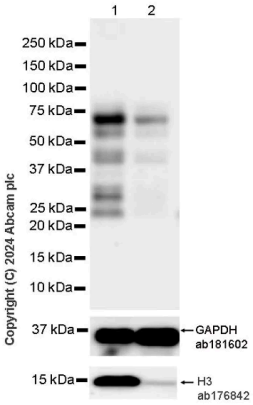
Lane 4:
Human tonsil tissue lysate at 20 µg

Lane 5:
Human liver tissue lysate at 20 µg

Secondary

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

Observed band size: 27 kDa, 15 kDa



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

The molecular weight observed is consistent with what has been described in the literature (PMID: 38240850).

The identity of the lower MW bands are unknown.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

All lanes:

Western blot - Anti-SOX4 antibody [EPR28132-51] (ab316850) at 1/1000 dilution

Lane 1:
DLD-1 (human colorectal adenocarcinoma epithelial cell) nuclear fraction at 20 µg

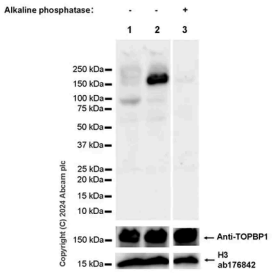
Lane 2:
DLD-1 cytoplasmic fraction at 20 µg

Secondary

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

Observed band size: 70 kDa, 36 kDa, 15 kDa

Exposure time: 59s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

The expression of TOPBP1 S1138 is upregulated in response to hydroxyurea treatment (PMID: 17446169).

Lysates were freshly made and used for Western blotting immediately to minimize protein degradation.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

In Western blot, Anti-TOPBP1 antibody staining at 1/1000 dilution.

All lanes:
Western blot - Anti-TopBP1 (phospho S1138) antibody [EPR27056-2] (ab316746) at 1/1000 dilution

Lane 1:
Untreated U-2 OS (human bone osteosarcoma epithelial cell) whole cell lysate (untreated membrane) at 30 µg

Lane 2:
U-2 OS treated with 2mM hydroxyurea for 24h whole cell lysate (untreated membrane) at 30 µg

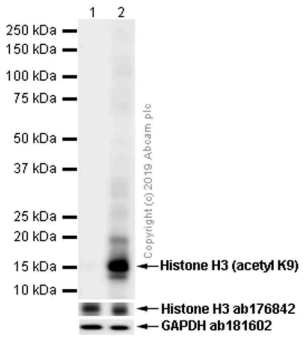
Lane 3:
U-2 OS treated with 2mM hydroxyurea for 24h whole cell lysate (alkaline phosphatase treated membrane) at 30 µg

Secondary

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

Observed band size: 170 kDa, 15 kDa

Exposure time: 92s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Blocking and diluting buffer and concentration: 5% NFDM/TBST. ab181602 was used as GAPDH loading control. ab176842 was for Histone H3 detection.
All lanes:
Western blot - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129) at 1/10000 dilution

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

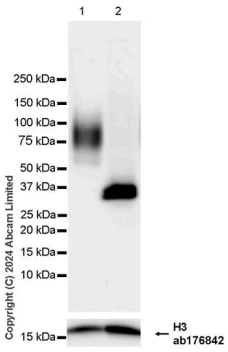
Lane 2:
HeLa (Human cervix adenocarcinoma epithelial cell) treated with 500ng/ml Trichostatin A for 4 hours whole cell lysate at 15 µg

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: 15 kDa



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

CD226 is a glycoprotein of approximately 60-90 kDa and detected as a 37-kDa band after treated with Peptide:N-glycosidase F (PNGase F).

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

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

All lanes:
Western blot - Anti-CD226 antibody [RM2068] (ab320009) at 1/1000 dilution

Lane 1:
Untreated HEL (human erythroleukemia erythroblast) whole cell lysate at 20 µg

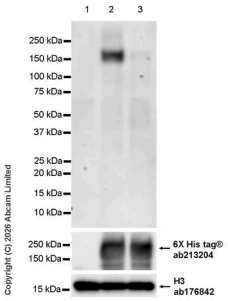
Lane 2:
HEL whole cell lysate treated with Peptide:N-glycosidase F (PNGase F) at 20 µg

Secondary

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

Observed band size: 37 kDa, 60-90 kDa, 15 kDa

Exposure time: 26s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

In Western blot, Anti-Histone H3 antibody [EPR16987] - Loading Control (ab176842) (1:100000) (36KDa); Anti-6X His tag® antibody [EPR20547] - ChIP Grade (ab213204) (1:5000)(150-250kDa).

All lanes:
Western blot - Anti-SOS1 (phospho Y1196) antibody [TF7-28-23] (ab325591) at 1/1000 dilution

Lane 1:
293T (human embryonic kidney epithelial cell) transfected with an empty vector containing a myc-His-tag® whole cell lysate at 20 µg

Lane 2:
293T transfected with a human wild-type SOS1 expression vector containing a myc-His-tag® whole cell lysate at 20 µg

Lane 3:
293T transfected with a human SOS1 (Y1196A mutation) expression vector containing a myc-His-tag® whole cell lysate at 20 µg

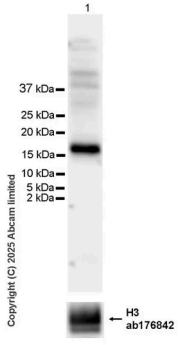
Secondary

All lanes:
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/10000 dilution

Developed using the ECL technique.

Observed band size: 152 kDa, 36 kDa, 150-250 kDa

Exposure time: 180s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining of HeLa (human cervical adenocarcinoma epithelial cell) histone fraction using rabbit Anti-Histone H3 antibody

Blocking and diluting buffer and concentration: 5% BSA/TBST.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) 1:100000 (15Kda).

All lanes:
Western blot - Anti-Histone H3.1 (asym-Di-methyl R17) antibody [EPR29607-55] (ab324457) at 1/1000 dilution

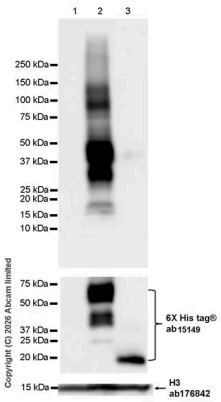
All lanes:
HeLa (human cervical adenocarcinoma epithelial cell) histone fraction at 20 µg

Secondary

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

Observed band size: 15 kDa

Exposure time: 8s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

The antibody is specific for the Pro-surfactant Protein B and does not recognize the mature form.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) 1:100000 (15Kda); Anti-6X His tag® antibody [EPR20547] - ChIP Grade (ab213204) 1:5000 (42Kda).

All lanes:
Western blot - Anti-Pro-surfactant Protein B (N-terminal) antibody [EPR30834-549] (ab327883) at 1/1000 dilution

Lane 1:
293T cells transfected with an empty vector containing a his-tag, whole cell lysate at 20 µg

Lane 2:
293T cells transfected with a human Pro-surfactant Protein B expression vector containing a his-tag, whole cell lysate at 1 µg

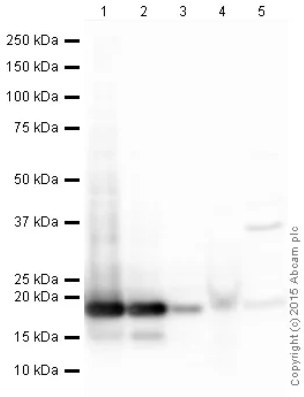
Lane 3:
293T cells transfected with a human mature SFTPB expression vector containing a his-tag, whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Observed band size: 42 kDa, 15 kDa

Exposure time: 1s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab176842 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution ab133406.
All lanes:
Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) at 1 µg

Lane 1:
HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate at 10 µg

Lane 2:
NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate at 10 µg

Lane 3:
Drosophila embryo nuclear extract (from melanogaster embryos 0-12hr) at 10 µg

Lane 4:
S.cerevisiae (Y190) Whole Cell Lysate at 20 µg

Lane 5:
S.pombe Whole Cell Lysate at 20 µg

Secondary

All lanes:
Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/50000 dilution

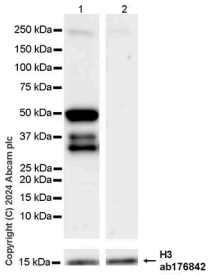
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa

Observed band size: 17 kDa

Exposure time: 5s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) was used as a loading control at a 1/100000 dilution.

Negative control: HeLa (PMID: 10492038).

The lower bands (~27–35 kDa) represent apoptosis-related cleavage fragments (PMID: 20395555; 32682001).

All lanes:
Western blot - Anti-MNDA antibody [253A] (ab188566) at 1/1000 dilution

Lane 1:
THP-1 (human monocytic leukemia monocyte) whole cell lysate at 20 µg

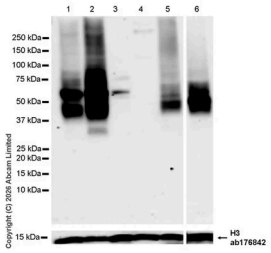
Lane 2:
HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/10000 dilution

Observed band size: 47 kDa

Exposure time: 37s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

Low expression: Hela, 293T.

Samples are non-boiled as boiling may cause protein aggregation.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) 1:100000 (15Kda).

All lanes:
Western blot - Anti-EBV Induced Gene 2/EBI2 antibody [EPR27339-553] (ab325923) at 1/1000 dilution

Lane 1:
HDLM-2 (human Hodgkin lymphoma cell) whole cell lysate at 50 µg

Lane 2:
MJ [G11] (human Peripheral blood T lymphoblast) whole cell lysate at 50 µg

Lane 3:
HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 50 µg

Lane 4:
293T (human embryonic kidney epithelial cell) whole cell lysate at 50 µg

Lane 5:
Human tonsil tissule lysate at 50 µg

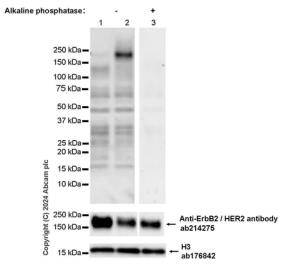
Lane 6:
Human lymph node tissule lysate at 50 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Observed band size: 42-70 kDa, 15 kDa

Exposure time: 81s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

ErbB2 / HER2 (phospho Y1221 + Y1222) expression can be induced by EGF treatment (PMID: 7728765).

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

In Western blot, Anti-ErbB2 / HER2 antibody - Total protein control (ab214275) staining at 1/1000 dilution.

All lanes:

Western blot - Anti-ErbB2 / HER2 (phospho Y1221 + Y1222) antibody [EPR28391-67] (ab316758) at 1/1000 dilution

Lane 1:
Untreated HeLa (human cervix adenocarcinoma epithelial cell) starved for 4 hours whole cell lysate (untreated membrane) at 20 µg

Lane 2:
HeLa starved for 4 hours, then treated with /ml EGF for 15 minutes whole cell lysate (untreated membrane) at 20 µg

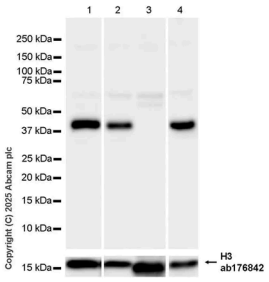
Lane 3:
HeLa starved for 4 hours, then treated with /ml EGF for 15 minutes whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

Secondary

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

Observed band size: 180 kDa, 15 kDa

Exposure time: 81s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

Low expression: Calu-3.

The identity of the bands higher than 50 kDa are unknown.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

All lanes:
Western blot - Anti-Aldolase C antibody [EPR26896-61] - C-terminal (ab323506) at 1/1000 dilution

Lane 1:
U-87 MG (human glioblastoma-astrocytoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
LNCaP (human prostate carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 3:
Calu-3 (human lung adenocarcinoma epithelial cell) whole cell lysate at 20 µg

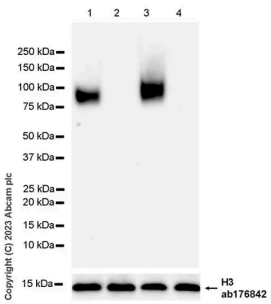
Lane 4:
RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Secondary

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

Observed band size: 39 kDa, 15 kDa

Exposure time: 8s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

In Western blot, ab315907 was shown to bind specifically to CD276.

Target of interest was observed at 90 KD in wild-type HEK293T cell lysates with no signal observed at this size in CD276 knockout cell line (lane 2, knockout cell line ab266658/ knockout cell lysate ab257097).

Lysates were freshly made, non-boiled and used immediately to minimize protein degradation.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

All lanes:
Western blot - Anti-B7-H3/CD276 antibody [RM2041] (ab315907) at 1/1000 dilution

Lane 1:
Wild-type HEK293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 2:
CD276 knockout HEK293T whole cell lysate at 20 µg

Lane 3:
LNCaP (human prostate carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
Raji (human Burkitts lymphoma B lymphocyte) whole cell lysate at 20 µg

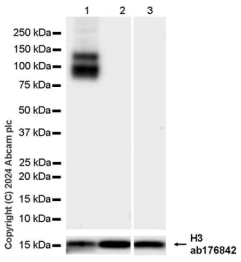
Secondary

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

Performed under reducing conditions.

Observed band size: 90 kDa, 15 kDa

Exposure time: 37s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

Negative control: Hela (PMID: 35164755), PANC-1

The molecular weight observed is consistent with what has been described in the literature (PMID: 36631513).

Samples are non-boiled as boiling may cause protein aggregation.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

All lanes:
Western blot - Anti-VE Cadherin antibody [RM2022] (ab318152) at 1/1000 dilution

Lane 1:
HUVEC (human umbilical vein endothelial cell) whole cell lysate at 20 µg

Lane 2:
HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

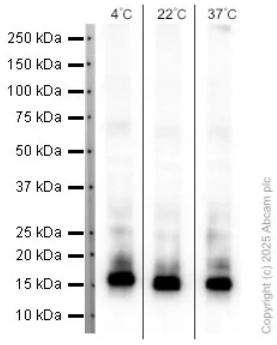
Lane 3:
PANC-1 (human pancreatic epithelioid carcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Observed band size: 75-120 kDa, 15 kDa

Exposure time: 15s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining of HeLa whole cell lysate using rabbit Anti-Histone H3 antibody

This blot was produced using 4-20% SDS-PAGE containing 15 µg of HeLa whole cell lysate per lane at 150V for 1hr before being transferred onto a 0.45 µm PVDF membrane at 75V for 1hr. The membrane was then blocked for 1hr using 5% NFDM/TBST, then incubated with ab176842 (1/2000) at room temperature for 1hr. After being washed three times in TBST, the membrane was incubated with Peroxidase conjugated goat anti-rabbit IgG (H+L) (ab97051) at 1/20,000 dilution for 1hr at room temperature. The membrane was washed three times again. Then the signal was developed using the ECL technique.

ab176842 was stored at a range of temperatures (+4°C, +22°C, +37°C) for 1 week before being tested in WB. The image shows the band intensity remains relatively constant across all storage temperatures, demonstrating that antibody activity is not affected under these conditions. This data was generated as part of a structured antibody stability study supporting Abcam’ s transition from cold⊠chain to ambient shipping.

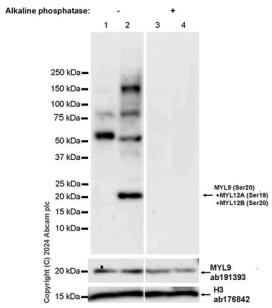
All lanes:
Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) at 1/2000 dilution

All lanes:
HeLa whole cell lysate at 15 µg with NDFM/TBST

Secondary

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

Exposure time: 3s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

The identity of the higher MW bands are unknown.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

In Western blot, Anti-MYL9 antibody [EPR13012 (2)] (ab191393) staining at 1/1000 dilution.

All lanes:
Western blot - Anti-MYL9 (pS20) + MYL12A (pS19) + MYL12B (pS20) antibody [EPR27203-65] (ab316750) at 1/1000 dilution

Lane 1:
Untreated HeLa (human epithelial cell line from cervical adenocarcinoma) whole cell lysate (untreated membrane) at 45 µg

Lane 2:
HeLa treated with 100ng/ml Calyculin A for 15 minutes, whole cell lysate (untreated membrane) at 45 µg

Lane 3:
Untreated HeLa (human epithelial cell line from cervical adenocarcinoma) whole cell lysate (alkaline phosphatase treated membrane) at 45 µg

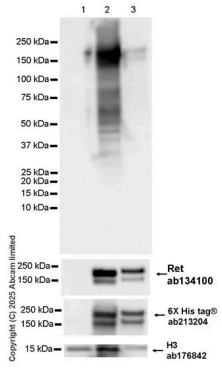
Lane 4:
HeLa treated with 100ng/ml Calyculin A for 15 minutes, whole cell lysate (alkaline phosphatase treated membrane) at 45 µg

Secondary

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

Observed band size: 20 kDa, 15 kDa

Exposure time: 70s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

The antibody shows weak reactivity with HEK293T cell lines overexpressing human Ret (Y1062A mutant)

In Western blot, Anti-Ret antibody (ab134100) (1:1000) (150KDa); Anti-6X His tag® antibody [EPR20547] - ChIP Grade (ab213204) (1:5000)(150KDa); Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) 1:100000 (15Kda).

All lanes:
Western blot - Anti-Ret (phospho Y1062) antibody [EPR30639-507] (ab326572) at 1/1000 dilution

Lane 1:

293T cells transfected with an empty vector containing a his-tag treated with 100uM pervanadate for 10 min whole cell lysate at 20 µg

Lane 2:
293T cells transfected with a human wild-type Ret expression vector containing a his-tag were treated with 100uM Pervanadate for 10 mins, whole cell lysate at 20 µg

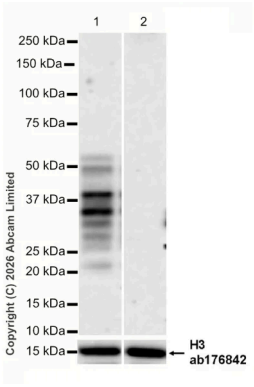
Lane 3:
293T cells transfected with a human Ret(Y1062A mutant) expression vector containing a his-tag were treated with 100uM Pervanadate for 10 mins, whole cell lysate at 2 µg

Secondary

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

Observed band size: 150 kDa, 15 kDa

Exposure time: 8s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

Performed under reducing conditions.

In Western blot, ab325636 was shown to bind specifically to RIPK3. Target of interest was observed at 37-50 kDa in wild-type THP-1 cell lysates (lane 1) with no signal observed at this size in RIPK3 knockout cell line (lane 2)(knockout cell line ab280065).

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) (1:100000) (17KDa).

All lanes:
Western blot - Anti-RIP3 antibody [RM1355] (ab325636) at 1/1000 dilution

Lane 1:
Wilde-type THP-1 (human monocytic leukemia monocyte) whole cell lysate at 20 µg

Lane 2:
Western blot - Human RIPK3 knockout THP-1 cell line (ab280065) at 20 µg

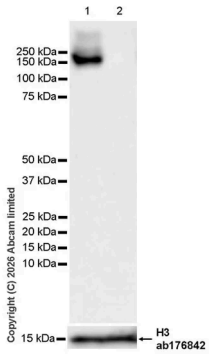
Secondary

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

Performed under reducing conditions.

Observed band size: 37-56 kDa, 17 kDa

Exposure time: 180s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

In Western blot, ab322735 was shown to bind specifically to ATP13A2. Target of interest was observed at 150 kDa in wild-type HEK-293 cell lysates (lane 1) with no signal observed at this size in ATP13A2 knockout cell line (lane 2)(knockout cell line ab274925)(knockout lysate ab274983)

Samples are non-boiled as boiling may cause protein aggregation.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) 1:100000 (15Kda).

All lanes:
Western blot - Anti-ATP13A2 antibody [MJF-D29756-28] (ab322735) at 1/1000 dilution

Lane 1:
HEK-293 (human embryonic kidney epithelial cell) whole cell lysate at 50 µg

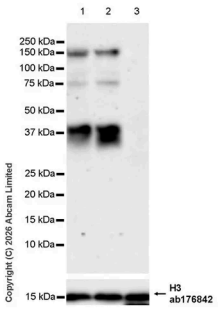
Lane 2:
Western blot - Human ATP13A2 knockout HEK-293 cell lysate (ab274983) at 50 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Observed band size: 150 kDa, 15 kDa

Exposure time: 92s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

Low expression: U-937 (PMID: 1384316).

Samples are non-boiled as boiling may cause protein aggregation.

The identity of the bands between 75 kDa and 150 kDa is unknown.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) 1:100000 (15Kda).

All lanes:
Western blot - Anti-CD72 antibody [EPR29739-526] (ab327352) at 1/1000 dilution

Lane 1:
Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate at 50 µg

Lane 2:
Ramos (human Burkitt's lymphoma B lymphocyte) whole cell lysate at 50 µg

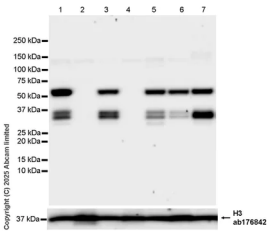
Lane 3:
U-937 (human histiocytic lymphoma monocyte) whole cell lysate at 50 µg

Secondary

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

Observed band size: 35-40 kDa, 15 kDa

Exposure time: 180s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

Performed under reducing conditions.

In Western blot, ab325424 was shown to bind specifically to SUFU. Target of interest was observed at 30, 35, 53 kDa in wild-type HAP1cell lysates (lane 1) and in wild-type HEK cell lysates (lane 3) with no signal observed at this size in SUFU knockout HAP1cell line (lane 2) and SUFU knockout HEK293T cell line (lane 4, knockout cell line ab267282 / knockout cell lysate ab257718).

The low MW band are due to degradation.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) 1:100000 (15Kda).

All lanes:
Western blot - Anti-SUFU antibody [EPR30547-60] (ab325424) at 1/1000 dilution

Lane 1:
Parental HAP1 (human chronic myelogenous leukemia near-haploid cell) whole cell lysate at 20 µg

Lane 2:
SUFU knockout HAP1 whole cell lysate at 20 µg

Lane 3:
Parental HEK293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 4:
SUFU knockout HEK293T whole cell lysate at 20 µg

Lane 5:
HEL (human erythroleukemia erythroblast) whole cell lysate at 20 µg

Lane 6:
293T whole cell lysate at 20 µg

Lane 7:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

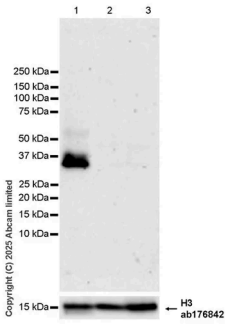
Secondary

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

Performed under reducing conditions.

Observed band size: 30 kDa, 35 kDa, 53 kDa, 37 kDa

Exposure time: 15s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining of SU-DHL-1 (human histiocytic lymphoblast-like cell) whole cell lysate using rabbit Anti-Histone H3 antibody

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

Low expression: MCF7, HeLa

Samples are non-boiled as boiling may cause protein aggregation.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) 1:100000 (15Kda).

All lanes:
Western blot - Anti-GPCR GPR97 antibody [EPR30227-586] (ab325332) at 1/1000 dilution

Lane 1:
SU-DHL-1 (human histiocytic lymphoblast-like cell) whole cell lysate at 40 µg

Lane 2:
MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate at 40 µg

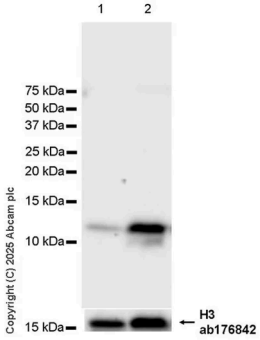
Lane 3:
HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 40 µg

Secondary

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

Observed band size: 38 kDa, 15 kDa

Exposure time: 180s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) staining at 1/100000 dilution.

All lanes:
Western blot - Anti-DAP12 antibody [EPR29151-41] (ab323318) at 1/1000 dilution

Lane 1:
Untreated THP-1 (human monocytic leukemia monocyte) whole cell lysate at 20 µg

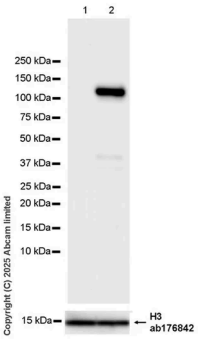
Lane 2:
THP-1 cells differentiated with TPA (12-O-Tetradecanoylphorbol-13-acetate) whole cell lysate at 20 µg

Secondary

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

Observed band size: 11 kDa, 12 kDa, 15 kDa

Exposure time: 26s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

Performed under reducing conditions.

In Western blot, ab324563 was shown to bind specifically to NEDD4. Target of interest was observed at 115 kDa in wild-type HAP1 cell lysates (lane 2) with no signal observed at this size in NEDD4 knockout cell line (lane 1) (lane 1, knockout cell line ab

ab256783).

To minimize protein degradation, cells/tissues were lysed immediately after harvest and then applied to a gel and transfer membrane for Western blotting as soon as possible.

The samples are unboiled.

All lanes:
Western blot - Anti-NEDD4 antibody [EPR30127-570] (ab324563) at 1/1000 dilution

Lane 1:
NEDD4 knockout parental HAP1 (human chronic myelogenous leukemia near-haploid cell) whole cell lysate at 20 µg

Lane 2:
Parental HAP1 whole cell lysate at 20 µg

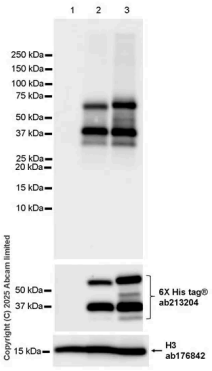
Secondary

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

Performed under reducing conditions.

Observed band size: 115 kDa, 15 kDa

Exposure time: 37s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

The antibody reacts with both the ROR gamma and ROR gamma t isoforms.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) 1:100000 (15Kda);
Anti-6X His tag® antibody [EPR20547] - ChIP Grade (ab213204) (1:5000)(37,55kDa).

All lanes:
Western blot - Anti-ROR gamma antibody [EPR27137-10] (ab325651) at 1/1000 dilution

Lane 1:
293T (human embryonic kidney epithelial cell) cells transfected with an empty vector containing a myc-His-tag® whole cell lysate at 20 µg

Lane 2:
293T cells transfected with human ROR gamma t expression vector containing a myc-His-tag® whole cell lysate at 20 µg

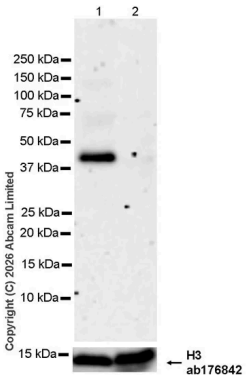
Lane 3:
293T cells transfected with human ROR gamma expression vector containing a myc-His-tag® whole cell lysate at 10 µg

Secondary

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

Observed band size: 37 kDa, 55 kDa, 15 kDa

Exposure time: 180s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining of Human skeletal muscle tissue lysate using rabbit Anti-Histone H3 antibody

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

Low expression: liver.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) 1:100000 (15Kda).

All lanes:
Western blot - Anti-MURF1 antibody [EPR29784-48] (ab327341) at 1/1000 dilution

Lane 1:
Human skeletal muscle tissue lysate at 50 µg

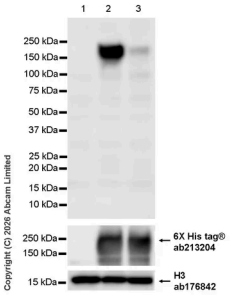
Lane 2:
Human liver tissue lysate at 50 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Observed band size: 40 kDa, 15 kDa

Exposure time: 180s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

In Western blot, Anti-Histone H3 antibody [EPR16987] - Loading Control (ab176842) (1:100000) (36KDa); Anti-6X His tag® antibody [EPR20547] - ChIP Grade (ab213204) (1:5000)(150-250kDa).

All lanes:
Western blot - Anti-SOS1 (phospho Y1196) antibody [TR16-7-3] (ab325592) at 1/1000 dilution

Lane 1:
293T (human embryonic kidney epithelial cell) transfected with an empty vector containing a myc-His-tag® whole cell lysate at 20 µg

Lane 2:
293T transfected with a human wild-type SOS1 expression vector containing a myc-His-tag® whole cell lysate at 20 µg

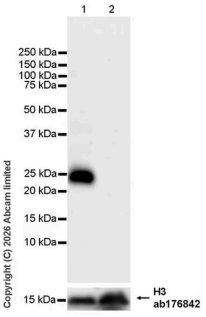
Lane 3:
293T transfected with a human SOS1 (Y1196A mutation) expression vector containing a myc-His-tag® whole cell lysate at 20 µg

Secondary

All lanes:
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/10000 dilution

Observed band size: 152 kDa, 36 kDa, 150-250 kDa

Exposure time: 3s



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Histone H3 Western blot staining using rabbit Anti-Histone H3 antibody

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

In Western blot, ab326362 was shown to bind specifically to CTSL. Target of interest was observed at 25 kDa in wild-type HEK-293T cell lysates (lane 1) with no signal observed at this size in CTSL HEK-293T knockout cell lysate(ab257213)/cell line (lane 2)(ab255449).

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) 1:100000 (15Kda).

All lanes:
Western blot - Anti-Cathepsin L/MEP antibody [EPR30887-518] (ab326362) at 1/1000 dilution

Lane 1:
Wild-type HEK-293T (human embryonic kidney epithelial cell) whole cell lysate at 80 µg

Lane 2:
Western blot - Human CTSL (Cathepsin L/MEP) knockout HEK-293T cell lysate (ab257213) at 80 µg

Secondary

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

Observed band size: 25 kDa, 15 kDa

Exposure time: 48s

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