abcam

ab322655

Anti-Histone H2A.X antibody [RM1271]

Recombinant | RabMAb | Advanced Validation | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Multiclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Clone number	RM1271
Purification technique	Affinity purification Protein A
Specificity	For WB, ab322655 showed weaker sensitivity to rat tissues, please adjust your sample quantity and protocol. Unsuitable for mouse/rat IP.
Concentration	0.512 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

PepArr

Tested

Species	Synthetic peptide
Dilution info	1/25000

Notes	-

Expected

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

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/2000
Notes	

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

Species	Mouse
Dilution info	1/4000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/4000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Species	Mouse
Dilution info	1/100
Notes	-

Species	Rat
Dilution info	1/100
Notes	-

Not recommended

Species	Synthetic peptide

Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50
Notes	-

Species	Mouse
Dilution info	1/50
Notes	-

Species	Rat
Dilution info	1/50
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Not recommended

Species	Mouse, Rat, Synthetic peptide
Dilution info	-

Notes	-

Target data

See full target information H2AX

Function	Variant histone H2A which replaces conventional H2A in a subset of nucleosomes. 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. Required for checkpoint-mediated arrest of cell cycle progression in response to low doses of ionizing radiation and for efficient repair of DNA double strand breaks (DSBs) specifically when modified by C-terminal phosphorylation.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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

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

View our range of recombinant multiclonal antibodies.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

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

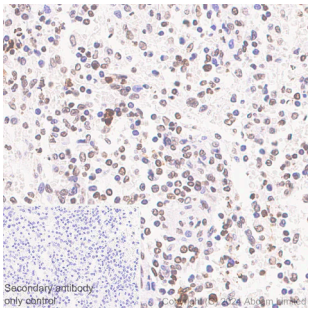
We do not recommend this combination. It is not covered by our product promise.

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.

21 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [RM1271] (ab322655)

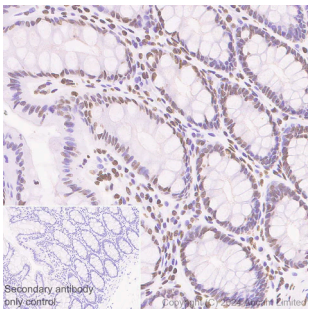
Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling Histone H2A.X with ab322655 at 1/2000 (0.256 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human spleen. The section was incubated with ab322655 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [RM1271] (ab322655)

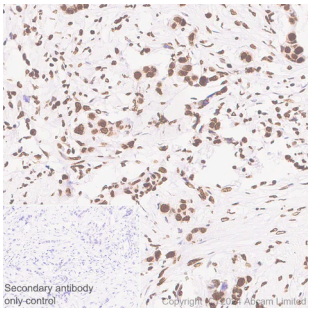
Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Histone H2A.X with ab322655 at 1/2000 (0.256 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human colon. The section was incubated with ab322655 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [RM1271] (ab322655)

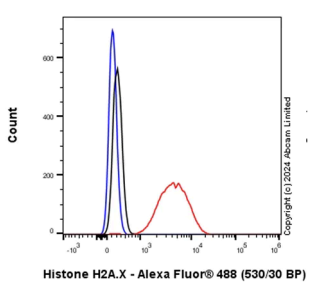
Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling Histone H2A.X with ab322655 at 1/2000 (0.256 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human breast carcinoma. The section was incubated with ab322655 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

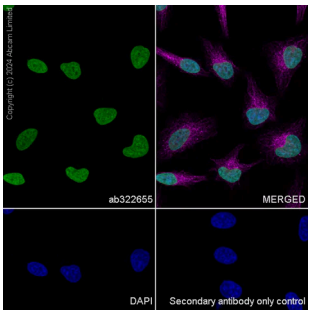
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Flow Cytometry (Intracellular) - Anti-Histone H2A.X antibody [RM1271] (ab322655)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervical adenocarcinoma epithelial cell) cells labelling Histone H2A.X with ab322655 at 1/50 dilution (1ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



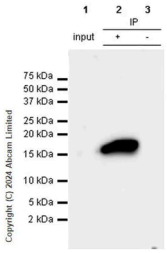
Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A.X antibody [RM1271] (ab322655)

Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized HeLa (human cervical adenocarcinoma epithelial cell) cells labelling Histone H2A.X with ab322655 at 1/100 (5.12 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2ug/ml dilution (Green).

Confocal image showing nuclear staining in HeLa cell line (shown in green). The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Magenta). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2ug/ml dilution.



Immunoprecipitation - Anti-Histone H2A.X antibody [RM1271] (ab322655)

Histone H2A.X was immunoprecipitated from 0.35 mg HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate with ab322655 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab322655 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate
Lane 2: ab322655 IP in HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab322655 in HeLa whole cell lysate

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

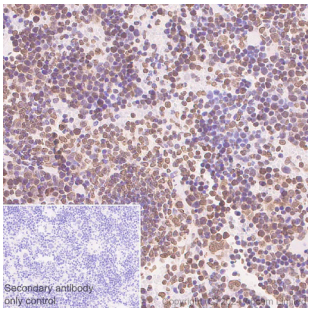
All lanes:
Immunoprecipitation - Anti-Histone H2A.X antibody [RM1271] (ab322655) at 1/30 dilution

All lanes:
HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 32s



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [RM1271] (ab322655)

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling Histone H2A.X with ab322655 at 1/4000 (0.128 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

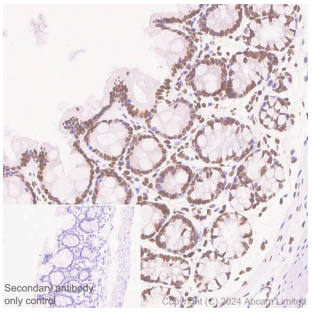
Positive staining on mouse spleen. The section was incubated with ab322655 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [RM1271] (ab322655)

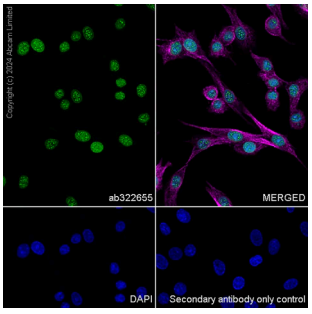
Immunohistochemical analysis of paraffin-embedded Rat colon tissue labeling Histone H2A.X with ab322655 at 1/4000 (0.128 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on rat colon. The section was incubated with ab322655 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



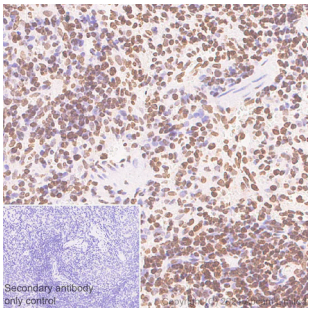
Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A.X antibody [RM1271] (ab322655)

Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized C6 (rat glial tumor glial cell) cells labelling Histone H2A.X with ab322655 at 1/100 (5.12 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2ug/ml dilution (Green).

Confocal image showing nuclear staining in C6 cell line (shown in green). The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Magenta). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2ug/ml dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [RM1271] (ab322655)

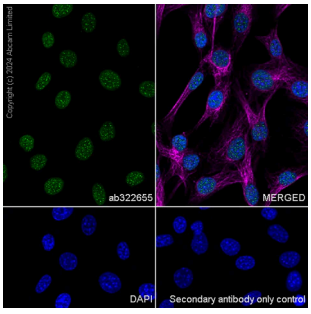
Immunohistochemical analysis of paraffin-embedded Rat spleen tissue labeling Histone H2A.X with ab322655 at 1/4000 (0.128 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on rat spleen. The section was incubated with ab322655 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



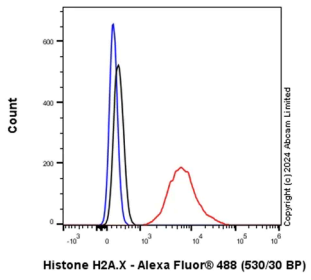
Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A.X antibody [RM1271] (ab322655)

Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labelling Histone H2A.X with ab322655 at 1/100 (5.12 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2ug/ml dilution (Green).

Confocal image showing nuclear staining in NIH/3T3 cell line (shown in green). The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Magenta). The Nuclear counterstain was DAPI (Blue).

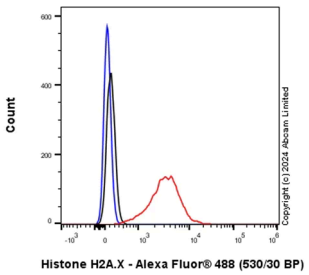
Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2ug/ml dilution.



Flow Cytometry (Intracellular) - Anti-Histone H2A.X antibody [RM1271] (ab322655)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labelling Histone H2A.X with ab322655 at 1/50 dilution (1ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

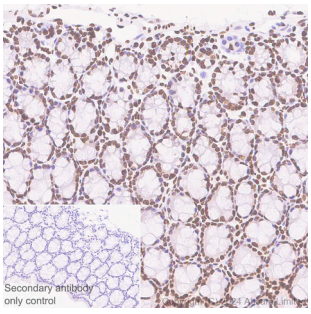
Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-Histone H2A.X antibody [RM1271] (ab322655)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized C6 (rat glial tumor glial cell) cells labelling Histone H2A.X with ab322655 at 1/50 dilution (1ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [RM1271] (ab322655)

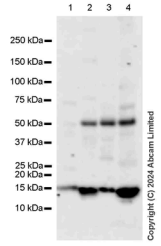
Immunohistochemical analysis of paraffin-embedded Mouse colon tissue labeling Histone H2A.X with ab322655 at 1/4000 (0.128 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on mouse colon. The section was incubated with ab322655 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Western blot - Anti-Histone H2A.X antibody [RM1271] (ab322655)

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

The identity of the higher MW band at approximately 50 kDa maybe IgG heavy chain.

All lanes:
Western blot - Anti-Histone H2A.X antibody [RM1271] (ab322655) at 1/1000 dilution

Lane 1:
Human cerebellum tissue lysate at 20 µg

Lane 2:
Human liver tissue lysate at 20 µg

Lane 3:
Human skeletal muscle tissue lysate at 20 µg

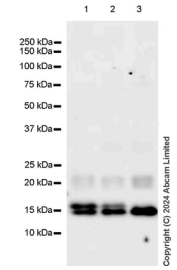
Lane 4:
Human spleen tissue lysate at 20 µg

Secondary

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

Observed band size: 15 kDa

Exposure time: 180s



Western blot - Anti-Histone H2A.X antibody [RM1271] (ab322655)

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

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 23109425).

All lanes:
Western blot - Anti-Histone H2A.X antibody [RM1271] (ab322655) at 1/1000 dilution

Lane 1:
THP-1 (human monocytic leukemia 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

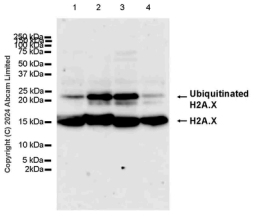
Lane 3:
RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Secondary

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

Observed band size: 15-20 kDa

Exposure time: 48s



Western blot - Anti-Histone H2A.X antibody [RM1271] (ab322655)

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

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 23109425).

All lanes:
Western blot - Anti-Histone H2A.X antibody [RM1271] (ab322655) at 1/1000 dilution

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

Lane 2:
293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

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

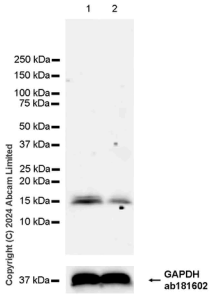
Lane 4:
PC-12 (rat adrenal gland pheochromocytoma cell) whole cell lysate at 20 µg

Secondary

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

Observed band size: 15-20 kDa

Exposure time: 180s



Western blot - Anti-Histone H2A.X antibody [RM1271] (ab322655)

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

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

All lanes:
Western blot - Anti-Histone H2A.X antibody [RM1271] (ab322655) at 1/10000 dilution

Lane 1:
293T (human embryonic kidney epithelial cell) transfected with scrambled siRNA control, whole cell lysate at 20 µg

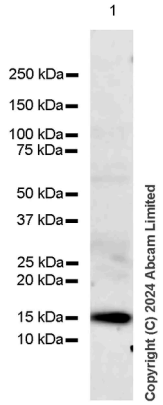
Lane 2:
293T transfected with siRNA specifically targeting Histone H2A.X, whole cell lysate at 20 µg

Secondary

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

Observed band size: 15 kDa, 17 kDa, 36 kDa

Exposure time: 180s



Western blot - Anti-Histone H2A.X antibody [RM1271] (ab322655)

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

All lanes:
Western blot - Anti-Histone H2A.X antibody [RM1271] (ab322655) at 1/1000 dilution

All lanes:
C6 (rat glial tumor glial cell) whole cell lysate at 20 µg

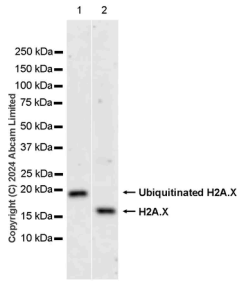
Secondary

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

Observed band size: 15 kDa

Exposure time: 180s

Western blot - Anti-Histone H2A.X antibody [RM1271] (ab322655)



Histone H2A.X Western blot staining using rabbit Anti-Histone H2A.X antibody

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

The expression profile/ molecular weight observed is consistent with what has been described in the literature

All lanes:
Western blot - Anti-Histone H2A.X antibody [RM1271] (ab322655) at 1/1000 dilution

Lane 1:
Mouse heart tissue lysate at 20 µg

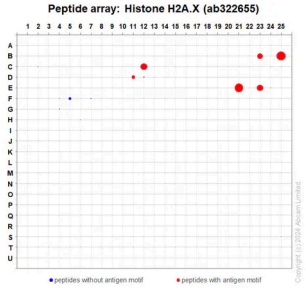
Lane 2:
Mouse spleen tissue lysate at 20 µg

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: 180s



Peptide Array - Anti-Histone H2A.X antibody [RM1271] (ab322655)

Peptide array analysis of ab322655 at 1:25000 (0.02 ug/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Fluo 647nm conjugated at 1:50,000 dilution.

ab322655 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).
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'.