abcam

Product datasheet

Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free ab215967

重组 RabMAb

15 References 13 图像

概述

产品名称	Anti-gamma H2A.X (phospho S139)抗体[EP854(2)Y] - BSA and Azide free
描述	兔单克隆抗体[EP854(2)Y] to gamma H2A.X (phospho S139) - BSA and Azide free
宿主	Rabbit
特异性	Unsuitable for mouse and rat IHC-P.
经测试应用	**适用于:** IHC-P, ICC/IF, Dot blot, WB, IP
种属反应性	**与反应:** Mouse, Rat, Human **预测可用于:** Sheep
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HepG2 cell lysate treated with etoposide; Jurkat cell lysate. IHC-P: Human kidney transitional cell carcinoma, human brain, human testis, human breast carcinoma, and human cervical carcinoma. ICC/IF: H2O2 treated HeLa cells. IP: HepG2 treated with etoposide and TSA whole cell lysate.
常规说明	ab215967 is the carrier-free version of **ab81299**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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. 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 production

For more information **see here**.

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

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C. Do Not Freeze.
存储溶液	pH: 7.20 Constituent: PBS
无载体	**是**
纯度	Protein A purified
克隆	单克隆
克隆编号	EP854(2)Y
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab215967**于以下的**经测试应用

"应用说明"**部分 下显示的**仅为**推荐的起始稀释度**;实际**最佳的稀释度**/浓**度**应**由使用者检定。**

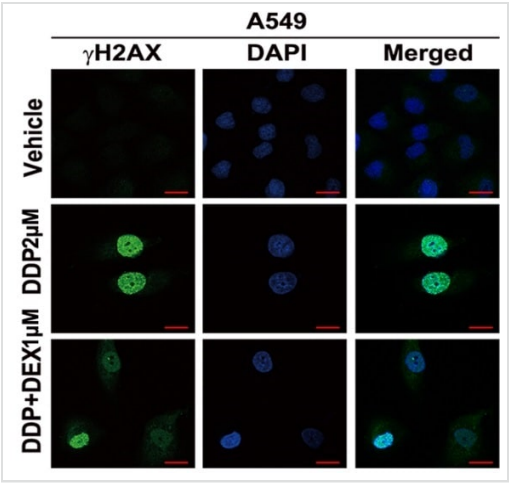
应用	Ab评论	说明
IHC-P		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
Dot blot		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 15 kDa.
IP		Use at an assay dependent concentration.

靶标

功能 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.

序列相似性	Belongs to the histone H2A family.
发展阶段	Synthesized in G1 as well as in S-phase.
结构域	The [ST]-Q motif constitutes a recognition sequence for kinases from the PI3/PI4-kinase family.
翻译后修饰	Phosphorylated on Ser-140 (to form gamma-H2AFX or H2AX139ph) in response to DNA double strand breaks (DSBs) generated by exogenous genotoxic agents and by stalled replication forks, and may also occur during meiotic recombination events and immunoglobulin class switching in lymphocytes. Phosphorylation can extend up to several thousand nucleosomes from the actual site of the DSB and may mark the surrounding chromatin for recruitment of proteins required for DNA damage signaling and repair. Widespread phosphorylation may also serve to amplify the damage signal or aid repair of persistent lesions. Phosphorylation of Ser-140 (H2AX139ph) in response to ionizing radiation is mediated by both ATM and PRKDC while defects in DNA replication induce Ser-140 phosphorylation (H2AX139ph) subsequent to activation of ATR and PRKDC. Dephosphorylation of Ser-140 by PP2A is required for DNA DSB repair. In meiosis, Ser-140 phosphorylation (H2AX139ph) may occur at synaptonemal complexes during leptotene as an ATM-dependent response to the formation of programmed DSBs by SPO11. Ser-140 phosphorylation (H2AX139ph) may subsequently occurs at unsynapsed regions of both autosomes and the XY bivalent during zygotene, downstream of ATR and BRCA1 activation. Ser-140 phosphorylation (H2AX139ph) may also be required for transcriptional repression of unsynapsed chromatin and meiotic sex chromosome inactivation (MSCI), whereby the X and Y chromosomes condense in pachytene to form the heterochromatic XY-body. During immunoglobulin class switch recombination in lymphocytes, Ser-140 phosphorylation (H2AX139ph) may occur at sites of DNA-recombination subsequent to activation of the activation-induced cytidine deaminase AICDA. Phosphorylation at Tyr-143 (H2AXY142ph) by BAZ1B/WSTF determines the relative recruitment of either DNA repair or pro-apoptotic factors. Phosphorylation at Tyr-143 (H2AXY142ph) favors the recruitment of APBB1/FE65 and pro-apoptosis factors such as MAPK8/JNK1, triggering apoptosis. In contrast, dephosphorylation of Tyr-143 by EYA proteins (EYA1, EYA2, EYA3 or EYA4) favors the recruitment of MDC1-containing DNA repair complexes to the tail of phosphorylated Ser-140 (H2AX139ph). Monoubiquitination of Lys-120 (H2AXK119ub) by RING1 and RNF2/RING2 complex gives a specific tag for epigenetic transcriptional repression. Following DNA double-strand breaks (DSBs), it is ubiquitinated through 'Lys-63' linkage of ubiquitin moieties by the E2 ligase UBE2N and the E3 ligases RNF8 and RNF168, leading to the recruitment of repair proteins to sites of DNA damage. Monoubiquitination and ionizing radiation-induced 'Lys-63'-linked ubiquitination are distinct events.
细胞定位	Nucleus. Chromosome.

图片



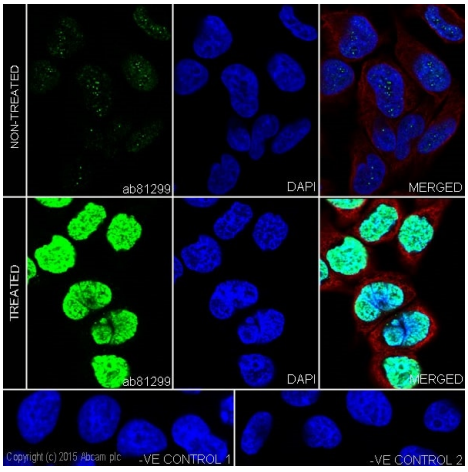
Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free (ab215967)

Immunofluorescence staining of A549 (Human lung carcinoma cell line) labeling gamma H2A.X (phospho S139) (green) with **ab81299**.

Cells were fixed in 4% paraformaldehyde for 15 minutes and permeabilized in PBS-0.2% Triton for 10 minutes. After blocked for 1 hour, primary antibody was diluted in blocking buffer (1/100) and incubated with fixed cells overnight at 4°C. Cells were washed and incubated with secondary antibodies (1/100) for 1 hour at room temperature. All slides were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Immunofluorescence was performed using confocal laser scanning microscopy (Lecia) or fluorescence microscopy (Olympus).

Dexamethasone, DEX; Cisplatin, DDP.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81299**).



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free (ab215967)

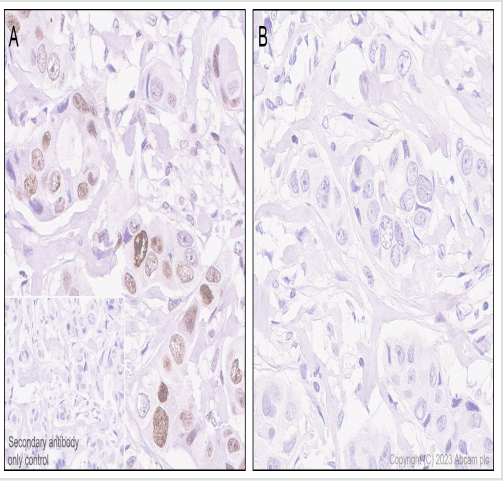
Immunocytochemistry/Immunofluorescence analysis of HeLa cells (untreated and treated with H_2O_2) labelling Histone H2A.X (phospho S139) with **ab81299** at 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. The cells were co-stained with **ab7291**, a mouse anti-tubulin (1/1000) using **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) as the secondary antibody. Nuclei counterstained with DAPI (blue).

Control 1: primary antibody (1/250) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).

This data was developed using the same antibody clone in a

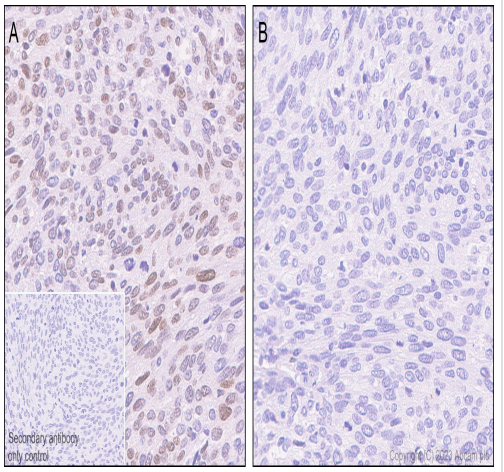
different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81299**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free (ab215967)

This data was developed using the same antibody clone in a different buffer formulation (**ab81299**).

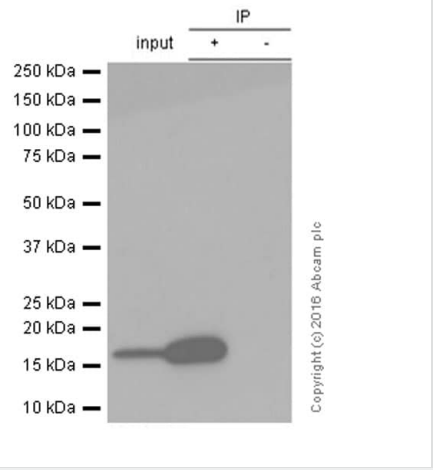
Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling gamma H2A.X with **ab81299** at 1/3000 (0.352 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on human breast carcinoma without lambda protein phosphatase treatment (image A). No signal was detected when tissues were treated with lambda protein phosphatase (image B). The section was incubated with **ab81299** for 15 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 **ab209101**. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free (ab215967)

This data was developed using the same antibody clone in a different buffer formulation (**ab81299**).

Immunohistochemical analysis of paraffin-embedded Human cervical carcinoma tissue labeling gamma H2A.X with **ab81299** at 1/3000 (0.352 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on human cervical carcinoma without lambda protein phosphatase treatment (image A). No signal was detected when tissues were treated with lambda protein phosphatase (image B). The section was incubated with **ab81299** for 15 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 **ab209101**. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.



Immunoprecipitation - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free (ab215967)

ab81299 at 1/40 immunoprecipitating Histone H2A.X (phospho S139) in HepG2 (human hepatocellular carcinoma epithelial) whole cell lysate observed at 15 KDa (lanes 1 and 2).

Lane 1 (input): HepG2 treated with etoposide and TSA whole cell lysate 10µg

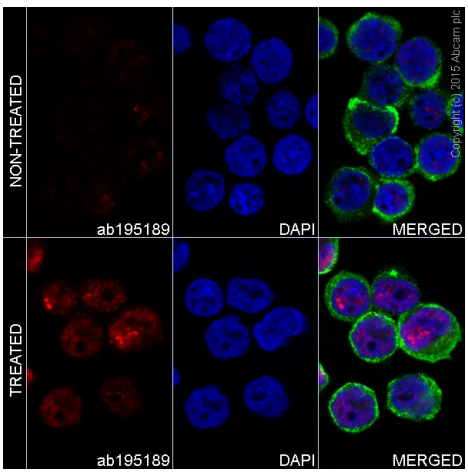
Lane 2 (+): **ab81299** + HepG2 treated with etoposide and TSA whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab81299** in HepG2 treated with etoposide and TSA

For western blotting, **ab81299** (Purified) at 1/200 dilution and VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

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

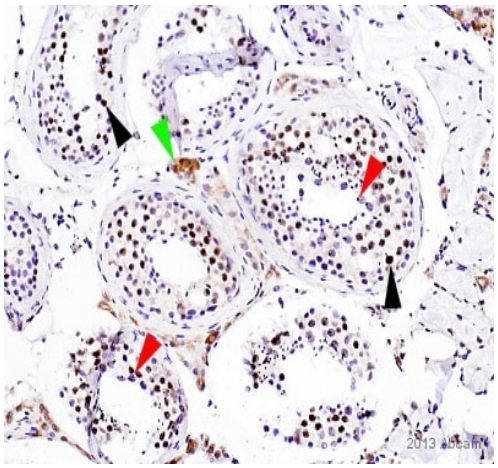
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81299**).



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free (ab215967)

Clone EP854(2)Y (ab215967) has been successfully conjugated by Abcam. This image was generated using Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (Alexa Fluor® 647). Please refer to **ab195189** for protocol details.

ab195189 staining Histone H2A.X in Jurkat cells. The cells were incubated with 25uM ETP for 5 hours (Treated) or solvent-only for control purposes (Non-treated). The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 10% normal goat serum in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab195189** at 1/200 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/200 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

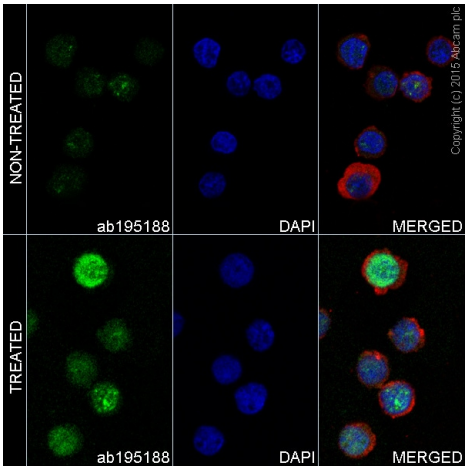


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free (ab215967)

This image is courtesy of an Abreview submitted by Carl Hobbs.

Ab81299 staining H2A.X in Human Testis tissue sections by Immunohistochemistry (Formalin/PFA fixed paraffin embedded sections). Tissue was fixed with formaldehyde and blocked with 1% BSA for 10 minutes at 21°C; antigen retrieval was by heat mediation in Citric acid. Samples were incubated with primary antibody (1/50 in TBS) for 2 hours at 21°C. A biotin conjugated Anti-Rabbit IgG (goat polyclonal) was used as the secondary antibody at a 1/250 dilution.

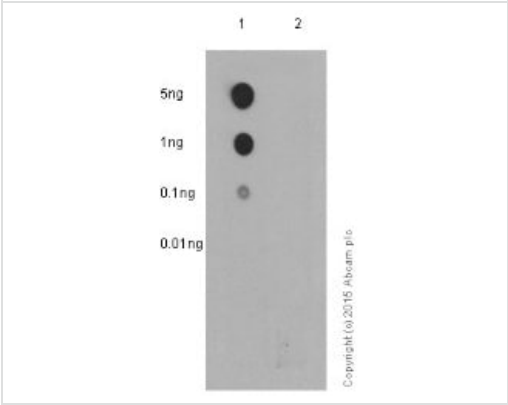
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81299**).



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free (ab215967)

Clone EP854(2)Y (ab215967) has been successfully conjugated by Abcam. This image was generated using Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (Alexa Fluor® 488). Please refer to **ab195188** for protocol details.

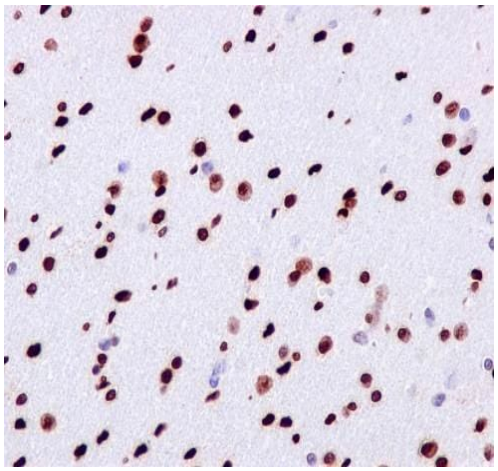
ab195188 staining Histone H2A.X in Jurkat cells. The cells were incubated with 25uM ETP for 5 hours (Treated) or solvent-only for control purposes (Non-treated). The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 10% normal goat serum in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab195188** at 1/50 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/200 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Dot Blot - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free (ab215967)

Dot blot analysis of Histone H2A.X single phospho peptide pS139 (lane 1) and Histone H2A.X non-phospho peptide (lane 2) with **ab81299** at 1/1000. Blocking and diluting buffer was 5% NFDM/TBST. The secondary antibody used was **ab97051** Peroxidase conjugated Goat Anti-Rabbit IgG, (H+L) at 1/100,000.

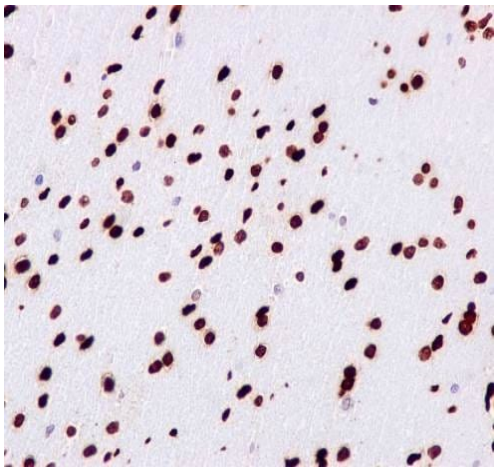
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81299**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free (ab215967)

Immunohistochemical staining of paraffin embedded human brain with unpurified **ab81299** at a working dilution of 1 in 50. The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0.

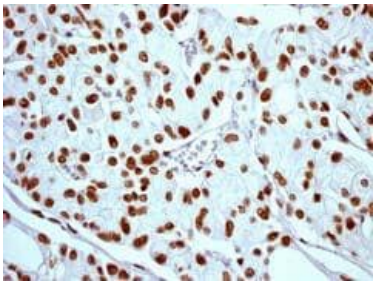
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81299**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free (ab215967)

Immunohistochemical staining of paraffin embedded human brain with purified **ab81299** at a working dilution of 1 in 100. The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81299**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free (ab215967)

Immunohistochemical analysis of formalin/PFA-fixed paraffin-embedded human kidney transitional cell carcinoma using unpurified **ab81299** at a dilution of 1/100.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81299**).

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - BSA and Azide free (ab215967)

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